

Exponent®



**Follow-Up Study Report:  
Oral Bioavailability of  
Dioxins/Furans in  
Tittabawassee River  
Floodplain Soil**



**Follow-Up Study Report:  
Oral Bioavailability of  
Dioxins/Furans in Tittabawassee  
River Floodplain Soil**

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## Acronyms and Abbreviations

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ANOVA	analysis of variance
CV	coefficient of variability
CYP1A1	cytochrome p-450 1A1
CYP1A2	cytochrome p-450 1A2
EROD	ethoxyresorufin O-deethylase
HR-GC/MS	high-resolution gas chromatography/mass spectrometry
1,4-HxCDF	1,2,3,4,7,8-hexachlorodibenzofuran
1,6-HxCDF	1,2,3,6,7,8-hexachlorodibenzofuran
MROD	methoxyresorufin O-deethylase
MSU	Michigan State University
NTP	National Toxicology Program
PCDD/F	polychlorinated dibenzo- <i>p</i> -dioxin/furan
1-PeCDF	1,2,3,7,8-pentachlorodibenzofuran
4-PeCDF	2,3,4,7,8-pentachlorodibenzofuran
RBA	relative bioavailability
RPD	relative percent difference
SOP	standard operating procedure
TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
TCDF	2,3,7,8-tetrachlorodibenzofuran

## Executive Summary

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This report presents the results of a follow-up to the pilot bioavailability study of Midland and Tittabawassee River floodplain soils (Exponent 2005). The objective of this follow-up study was to repeat the pilot oral bioavailability study in rats, with study design modifications structured to allow an assessment of the possible impact of observed differential enzyme induction on the estimation of relative bioavailability of selected dioxins and furans of importance from a soil sample from the Tittabawassee River floodplain. This follow-up was motivated by the findings of the pilot study, which showed:

1. Statistically significant differences between RBA estimates derived from rats compared to swine, and
2. A markedly higher RBA estimate for TCDF than for the other congeners.

These differences were hypothesized to be due to the observed differential induction of hepatic EROD activity (a marker for CYP1A1 induction) between the rats dosed with soils and their respective dose-matched reference groups (matched on an *administered* dose basis), with higher enzyme activity observed in the reference-group rats compared to the rats in the respective soil groups. CYP1A1 is directly involved in the metabolism of TCDF, and its role in the metabolism of other furan congeners was unknown.

This follow-up study was conducted with the same floodplain soil sample as used in the pilot study (Table 1) and multiple oil reference groups, with administered doses of the five furan congeners that contribute most to the soil TEQ matched to 0.2, 0.5, and 0.8 times the administered dose in soil. The range of oil reference doses was selected with the goal of matching hepatic TEQ (i.e., the *absorbed* dose) and EROD activity between at least one oil reference group and the soil group. The test materials were administered daily to rats for 30 days, and at the end of the study, the fraction of the total administered dose of each congener remaining in the liver and adipose tissue of each study animal was quantified.

The specific research objectives of this study were to:

1. Evaluate hepatic EROD and MROD activity as a function of hepatic TEQ concentration in the tested dose range
2. Assess any dose dependency of the elimination rate for each congener by examining the fraction of administered dose retained across dose rates and as a function of EROD activity, MROD activity, and hepatic TEQ concentration
3. Base a revised RBA calculation on the oil reference group(s) that match the soil group on hepatic TEQ and EROD activity, and compare the results to the original pilot-study results for rats and swine.



The results of the follow-up study demonstrated:

1. A clear relationship between hepatic TEQ and both EROD and MROD activity in the liver of the study animals, although the effect of hepatic TEQ on EROD activity was stronger
2. A clear impact of both hepatic TEQ concentration on the fraction of administered dose retained in the animal tissues for four of the five compounds, and a strong effect of and hepatic EROD (but not MROD) activity on the retention of TCDF, but not the other compounds.

These findings indicate that calculation of relative bioavailability of compounds in the soil, compared to the same compounds administered in corn oil, requires the use of an oil reference group that is matched both on hepatic TEQ and on hepatic EROD activity. In this study, the oil reference groups given doses of 0.5 and 0.8 times that in the soil group provided adequate comparison groups for calculation of RBA.

Based on those oil reference groups, the RBA of each of the five predominant floodplain furan congeners was estimated. The estimated RBAs for all five congeners were between 55% and 65%, with a TEQ-weighted RBA estimate of 58% to 60% for the floodplain soil compared to the oil reference groups with matched hepatic TEQ and EROD activity. In comparison with the results of the pilot study:

- The RBA estimates were similar to those obtained in rats in the pilot-study phase for all congeners except TCDF. The marked elevation of apparent RBA of TCDF, compared to the other furan congeners, observed in the pilot study was not observed when the hepatic TEQ and EROD activity were matched between the oil reference group and the soil group.
- The RBA estimates obtained in the follow-up study using rats remained statistically significantly higher than those obtained using swine during the pilot study. The difference in RBA estimates between species may represent differences due to the mode of soil administration (soil mixed with feed in the rats vs. administration of soil in wrapped in dough balls for the swine) or may represent true species differences in bioavailability of the furan compounds in this soil.

The pilot study and the follow-up study were undertaken to demonstrate and test a methodology to evaluate relative bioavailability of dioxin and furan congeners in soils containing mixed dioxin and furan congeners. Based on the results of these two studies, it does appear possible to use the mass-balance approach envisioned here to assess the bioavailability of soils with these compounds in the concentration range relevant to the Midland and Tittabawassee River floodplain soil contamination. However, the follow-up study in rats demonstrated clear relationships between the elimination rate of four of the five tested congeners and hepatic TEQ and EROD activity in the tested dose ranges. Any further studies should take steps to match the reference and soil groups on these parameters, probably by using a range of oil reference dose groups at fractions of the total soil dose, as demonstrated in the follow-up study.

Another key conclusion is that there appear to be true species differences in relative oral bioavailability between rats and swine. Such species differences have been observed for other classes of compounds in soil. The relevant question is which species provides a more representative model of the human gastrointestinal tract, but an assessment of this question is beyond the scope of this report.

If further bioavailability testing of soils is conducted, several additional minor modifications to the study protocol could be made to provide additional relevant information or to reduce costs:

1. Consider addition of hepatic CYP1A2 protein determination. Hepatic sequestration of the furan congeners was dose-related, even over the relatively narrow dose range used in this study, and may indicate some induction of CYP1A2 protein, even though the changes in MROD activity observed in this study were very slight.
2. Use composite tissue samples from within each oil reference group to obtain a single hepatic and adipose tissue sample for HR/GC-MS analysis for each group. The variability in tissue concentrations within these groups was consistent and relatively minor between the pilot and follow-up study, and continued use of individual tissue analyses among animals in these dose groups is probably unnecessary.
3. Consider analysis only for a single furan congener from the floodplain soils. Use of the range of oil reference doses and resulting matching on hepatic TEQ and EROD activity produced very consistent bioavailability estimates across congeners. If only a single furan congener (probably 4-PeCDF) were used as a marker for bioavailability, this would reduce analytical costs but would still provide a reasonable surrogate for the other furan congeners.

## Introduction

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The objective of this follow-up study was to repeat the pilot rat oral bioavailability study (Exponent 2005), with certain study design modifications (Appendix A). These modifications are structured to allow an assessment of the possible impact of differential enzyme induction on the estimation of relative bioavailability of selected dioxins and furans of importance from a soil sample from the Tittabawassee River floodplain. This follow-up was motivated by the findings of the pilot study that showed statistically significant differences in hepatic ethoxyresorufin O-deethylase (EROD) activity (a marker for cytochrome P450 1A1 induction) between the rats dosed with soils and their respective reference groups (congener-matched administered doses), with higher enzyme activity observed in the reference-group rats compared to the rats in the respective soil groups.

The observed differences in EROD activity were likely due to a difference in absorbed dose of dioxin and furan (PCDD/F) compounds, which led to statistically significantly different hepatic TEQ concentrations. The higher EROD activity in the reference groups compared to the soil groups was likely due to higher liver TEQ concentrations achieved in the reference groups due to higher absorbed doses of PCDD/Fs, and the resulting increased hepatic EROD activity.

CYP1A1 is responsible for the metabolism of 2,3,7,8-TCDF in rats (Tai et al. 1993), and induction of CYP1A1 has been shown to strongly increase the hepatic metabolism rate for TCDF in rats (McKinley et al. 1993; Olson et al. 1994). 4-PeCDF also can induce its own metabolism due to induction of CYP1A enzymes (Brewster and Birnbaum 1987). Other compounds, including TCDD and 1-PeCDF, show decreased retention of administered dose with increasing dose in subchronic studies, suggesting autoinduction of metabolism, although the specific metabolic pathways have not been identified (DeVito et al. 1998; Diliberto et al. 2001; Jackson et al. 1998). The metabolic pathways for the other compounds that contribute substantially to the total TEQ in the Midland and Tittabawassee River floodplain soils have not been examined to date but may be influenced by CYP1A1 induction. Distribution and retention of PCDD/F congeners can also be influenced by induction of hepatic CYP1A2 protein, which acts as a binding protein for these congeners (Diliberto et al. 1999).

Because the method used to estimate relative bioavailability in this study relies on an assumption that the elimination rate (including elimination through metabolism and other clearance mechanisms) for each compound is the same in the soil and oil reference groups, demonstrated statistically significant differences in EROD activity (a marker for CYP1A1) among the groups may result in invalid estimates of relative bioavailability for any congener for which metabolism is mediated by CYP1A1. In the pilot study, estimates of relative bioavailability for many of the compounds in the study were statistically significantly different between the rats and the swine. The rats displayed different EROD activities in the soil and reference groups (while the swine did not); therefore, this factor may account for some of the observed differences in apparent relative bioavailability between the two species. Other factors related to differing tissue concentrations, including differential rates of passive elimination at different liver or body concentrations, could also confound the interpretation of the initial pilot study results. Therefore, the goal of this effort was to match *absorbed* doses (as opposed to

*administered* doses) of congeners for which inducible metabolism may be affecting the interpretation of the results from the pilot study. Dose levels for the oil reference groups were selected so as to 'bracket' the likely absorbed dose from soil.

This follow-up study was conducted with the same floodplain soil sample that was used in the pilot study (Table 1) and multiple oil reference groups, with administered doses of the five furan congeners that contribute most to the soil TEQ matched to 0.2, 0.5, and 0.8 times the administered dose in soil. The range of oil reference doses was selected with the goal of matching hepatic TEQ and EROD activity between at least one oil reference group and the soil group. This approach was used to address the following research objectives:

1. *Evaluate EROD/MROD activity as a function of hepatic TEQ.* EROD and methoxyresorufin O-deethylase (MROD) activities for all individual animals and dose groups will be plotted versus hepatic TEQ concentration. The hepatic concentration-response curves for EROD and MROD activity will be characterized. The oil reference group(s) that provide the closest match to the hepatic TEQ, EROD, and MROD activity of the soil group will be identified.
2. *Assess any dose dependency of elimination rate by congener.* Liver and adipose tissue concentration data from each animal in each of the three oil reference groups will be analyzed to estimate the fraction of total administered dose retained in the tissues at the end of the 30-day dosing period for each of the five target congeners. If there is no dose dependence of elimination rate for a given congener, the fraction of administered dose retained should be similar among all oil reference groups regardless of administered dose. If the fraction of administered dose retained decreases or increases with increasing administered dose, this would provide evidence that the elimination rate of this congener is dose dependent in the range of doses examined.
3. *Calculate RBA for the congeners in soil based on matched hepatic TEQ and EROD activity.* The relative bioavailability of the congeners in soil will be estimated using the same calculation procedures outlined in the pilot-study report. However, these calculations will be presented based only on the one or two oil reference group(s) with hepatic TEQ and EROD activities that are most similar to those of the soil group, as identified in step 1 above. The results will be compared to those obtained in the original pilot study for both rats and swine, to evaluate the consistency of results between trials and to assess whether the estimates based on rat as the experimental model, once adjusted for enzyme induction, become more consistent with the results obtained using swine.

## Methods and Materials

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In general, the methods used in this study are similar to those in the pilot study (Exponent 2005), with modifications as described in Appendix A. These methods are described below.

### Dose Preparation and Administration

The test soil (sample THT02769, <250- $\mu$ m size fraction) was blended with PMI Nutrition International, Rodent LabDiet<sup>®</sup> 5001 (meal) (5% w/w) at WIL Research Laboratories, Inc. (WIL) in Ashland, Ohio. The WIL report describing the diet blending is provided in Appendix B, and results for concentrations of PCDD/Fs in the Rodent LabDiet<sup>®</sup> batch used in this study are provided in Table 2. To accomplish the blending of soil into the rat diet, soil (250 g) and diet (1,000 g) were blended in a Hobart mixer for 5 minutes to create a diet pre-mixture. The pre-mixture was then blended with 3,750 g of diet in a V-blender to create the final 5,000-g diet batch. Diet homogeneity samples (100 g) were collected from the initial, middle, and final material that emerged from the V-blender; these samples were sent to Alta for analysis of PCDD/F concentrations. Results for the pre-dosing soil/diet mixture (Table 3) show that the five most important congeners were recovered with coefficients of variability (CVs) ranging from 6.7% to 11%. These measurements of blended diet PCDD/F concentrations and homogeneity were considered acceptable to proceed with the study.

The three gavage reference materials for the rat study were prepared in corn oil/acetone (99:1), and were designed to deliver dioxin/furan doses that would achieve administered daily doses equal to 0.2, 0.5, and 0.8 times the administered doses in the soil/feed mixture. To create these reference mixtures, the five dioxin/furan congeners that contribute most to TEQ in the soil sample were spiked into acetone (10 mL), and the concentrations of the five congeners in the spiked acetone were measured to confirm that analytical concentrations were close to target concentrations. Subsequently, 4 mL of this acetone was added to 396 mL of corn oil (Spectrum Chemicals & Laboratory Products, National Formulary [NF] grade; analysis of the corn oil indicated negligible dioxin/furan concentrations [Table 2]). The three corn-oil/acetone reference materials were then assayed for concentrations of the five target congeners (Table 4). Relative percent differences (RPDs) between target and pre-dosing measured concentrations ranged from 0.9% to 14%. These results were considered acceptable for use in the study. The gavage reference mixtures were stored in amber glass bottles sealed with Teflon-lined lids, and were used within 60 days of preparation.

### Animal Handling and Dosing

Animal handling and dosing during the rat follow-up study were performed as described in the pilot study report (Exponent 2005), with modifications as described in the follow-up study design document (see Appendix A), a brief summary of which follows.

Thirty-eight 4-month-old female Sprague-Dawley rats, weighing between 250 and 290 g, were obtained from Harlan (Indianapolis, Indiana) and placed in individual stainless-steel cages. Each rat was weighed two days after arrival (Day -5) (during the quarantine period) and on Day 1 of the dosing period, and then weekly until study termination. The rats were provided with PMI Nutrition International Rodent LabDiet<sup>®</sup> 5001 (meal) and de-ionized water *ad libitum* during the one-week quarantine period, and their health status was monitored. All LabDiet<sup>®</sup> 5001 fed to the rats (including during the quarantine period and to the oil reference groups during the dosing period) was from the same batch of LabDiet<sup>®</sup> 5001 that was used by WIL Research to prepare the blended rat diets (Table 2). Five days prior to the start of dosing, healthy animals were assigned randomly to six dose groups (five rats/group for animals not being gavaged; seven rats/group for animals being gavaged; dose groups are identified in Table 5). Based on gavage-related mortality observed in the pilot study, seven (rather than five) were included in each of the oil reference groups during the compound administration phase of the study, to ensure that at least five animals reach the conclusion of the 30-day dosing period. At the end of the administration period, five rats were selected at random from all surviving rats in each gavage group for tissue collection.

During the 30-day dosing period, each rat received 50 g of feed every 2 days (clean feed for Groups 1–5, and feed/soil mixture for Group 6). The weight of any unconsumed feed at the end of each 2-day period was measured, and an estimate was made of the weight of any spilled feed. Dose groups 2–5 were gavaged daily with 1 mL of the corn-oil (for Group 2) or corn-oil/acetone reference mixtures (for Groups 3–5).

Twenty-four hours after the last dose was administered, the rats were weighed and terminated under CO<sub>2</sub> anesthesia. Their livers were excised, blotted dry, weighed, and wrapped in foil. The liver samples for the EROD and MROD assays were collected (1-g samples) from the livers of each rat. The sample was minced, placed in a 2-mL cryovial, immediately frozen in liquid nitrogen, and sent to Entrix for analysis. The remainder of the liver tissue was then frozen and shipped to Alta for the analytical work. For Groups 2–6, analyses were performed on each individual liver sample. For the control groups 1 and 2, a composite liver sample was created for analysis by compositing equal amounts of liver sample from each of the five animals in the group. As much fatty tissue as possible (3–6 g) was collected from within the abdominal cavity of each rat, weighed, and wrapped in foil. The fat samples were frozen and shipped to Alta for the analytical work. For the control groups 1 and 2, a composite adipose sample was created for analysis by compositing equal amounts of fatty tissue from each of the five animals within the group.

A 75-g post-dosing subsample of the blended rodent diet was collected and shipped to Alta for analysis of dioxins/furans, to evaluate the stability of the blended diet during the 30-day dosing period, and to confirm the doses of dioxins/furans delivered to the rats (Table 3). The CV among congener concentrations in all four samples of the blended rodent diet (three pre-dosing and one post-dosing) was no greater than 13% for any congener detected above the lower calibration limit, indicating that the diet was stable during the study. In addition, the gavage reference mixtures were shipped to Alta for post-dosing analysis (Table 4). The CV between congener concentrations in the pre- and post-dosing gavage reference mixtures was no greater than 17%, with nearly all below 10%, indicating that the reference mixtures were also stable during the study period.

Two rats, #25 (Group 2) and #52 (Group 5), did not complete the 30-day dosing period. These were sacrificed before study completion because of poor feed intake. On necropsy, they were diagnosed as having aspiration pneumonia. An additional six rats were randomly excluded from the group of animals used for tissue collection, as described above.

Rat carcasses from the follow-up study were wrapped in foil, placed in individual labeled zipper-sealed freezer bags, and archived ( $-80^{\circ}\text{C}$ ) for possible further analysis.

## Tissue Sample Homogenization and Analysis for EROD/MROD Activity and PCDD/F Concentrations

At Entrix, liver microsomes were prepared from each liver sample, and the protein levels and enzymatic activities were measured according to the MSU Standard Operating Procedure (SOP) No. 250 (v 1.1), titled *Protocol for Liver Microsome Preparation, and Microsomal Protein Measurement and AROD Assays in the same 96-Well Plate*. EROD/MROD activities and protein concentrations were measured fluorometrically at the end of the assay, using a Cytofluor multiplate reader (Appendix C).

At Alta, the rat liver samples were homogenized using a Cuisinart mini-prep processor. The processor was run on the “high” setting until the sample was liquefied (for the liver samples) or thoroughly homogenized (for the fat samples). The sample was then poured into separate 40-mL amber glass VOA vials for extraction. After homogenization of each sample, all parts of the processor that were in contact with sample material were washed with soap and hot water, rinsed with de-ionized water, and then rinsed with ultra-high-purity solvents (hexane followed by dichloromethane).

The rat fat samples were homogenized with a Sumeet Multi-Grind Model 964, which is a small-volume grinder that is suitable for small sample sizes. Samples were collected directly from the grinder into labeled amber glass jars. Between samples, all stainless-steel parts of the grinder that were in contact with sample material were washed with soap and hot water, rinsed with de-ionized water, and then serially rinsed with ultra-high-purity solvents (acetone, toluene, hexane, and dichloromethane). The polycarbonate grinder lid was washed with soap and hot water, rinsed with de-ionized water, and then serially rinsed with ultra-high-purity methanol followed by hexane.

Subsamples of the liver and fat homogenates were extracted in methylene chloride/hexane and analyzed for lipid content (EPA Method 1613), and PCDD/F concentrations by HR-GC/MS (EPA Method 1613).

## Data Analysis

The EROD and MROD activities were analyzed as follows:

- The hepatic TEQ concentrations and levels of EROD and MROD activity among dosing groups were compared using an analysis of variance (ANOVA)

followed by Dunnett's multiple comparison test at an overall 95% confidence level, to identify the oil reference group or groups with hepatic TEQ and EROD and MROD activities that are not statistically significantly different from those of the soil group.

- The relationship between measured EROD and MROD activity and hepatic TEQ concentration among all experimental animals was assessed using linear regression to evaluate whether a statistically significant relationship between enzyme activity and hepatic TEQ was present.

The mass of each congener retained at the end of 30 days in the liver and adipose tissue in each animal was estimated by multiplying the tissue concentration by the measured organ weight (liver) or the estimated adipose tissue weight (estimated as a function of body weight at sacrifice using the method of Bailey et al. 1980, as reported by Brown et al. 1997). This estimated retained mass was compared to the total administered dose over 30 days to obtain the fraction of total administered dose retained by each animal at the end of 30 days.

The fraction of administered dose retained for each congener was evaluated for all individual animals across oil reference groups using multivariate linear regression (least squares) to identify any relationship between fraction retained and hepatic TEQ concentration, EROD activity, or MROD activity. Among the oil reference-treated animals, a statistically significant relationship between the fraction of any specific congener retained and the enzyme activity or hepatic TEQ concentration would indicate a dependency of elimination rate on that parameter for that congener.

## Estimation of Relative Bioavailability

Relative bioavailability was estimated by comparing the fraction of administered dose retained in the tissues of animals in the groups dosed with soil with the fraction of administered dose retained by animals given a reference corn-oil solution, similar to the method used by Wittsiepe et al. (2004). The mathematical basis for the calculation is described in detail in the Exponent (2005) report on the pilot bioavailability study. As described in that report, this method relies on two key assumptions:

1. Elimination rates of the study congeners would be the same between the soil and oil reference groups, and
2. The majority of retained administered dose would be distributed in liver and adipose tissues, and the proportion of retained dose distributed to tissues other than liver and adipose would not be different in soil-dosed groups compared to oil reference-dosed groups.

If these two assumptions hold, the relative bioavailability of each congener in the soil group can be estimated by comparing the fraction of administered dose of that congener in the soil group ( $FR_{soil}$ ) to the comparable fraction retained in the oil reference group ( $FR_{ref}$ ):



$$RBA = \frac{FR_{soil}}{FR_{ref}} \quad (\text{Eq. 1})$$

Because of the differential hepatic EROD activity among experimental groups observed in the pilot study (Exponent 2005), the methods in this follow-up study were modified to use multiple oil reference dosing groups at varying fractions of the administered soil dose, as described above, resulting in at least one oil reference group with hepatic EROD activity and TEQ concentrations not significantly different from the soil group. Relative bioavailability of the congeners of interest in the soil was assessed by comparing the fraction retained between the soil group and the oil reference group or groups with the best-matched EROD activity and hepatic TEQ concentration. A TEQ-weighted estimate of relative bioavailability for the soil sample was estimated by weighting the individual congener bioavailability estimates by their respective percent contribution to the TEQ concentration of the soil sample.

## Results

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At the end of the administration period, five rats were selected at random from all surviving rats in each oil reference group for tissue collection. Tissue was collected from all five rats in the soil group and feed control group. As discussed in the Animal Handling and Dosing section, two rats from the oil reference groups (one each from Groups 2 and 5) were sacrificed before the end of the study because their feed intake had dropped significantly. Results from the rats that were sacrificed early or were randomly excluded were not included in the data analysis discussed below. Detailed study data are presented in Appendix D.

### Feed Intake

Details of feed intake for all groups are presented in Table D-1, and the feed intake is illustrated in Figure 1. The mean daily feed intake for all dosing groups was approximately 15 g/day. The mean daily feed intake for the Tittabawassee River soil group was 18 g/day (Group 6), and was 17 g/day for the feed control group. The oil control and one of the oil reference groups (Groups 2 and 3) had a mean intake of 13 g/day, and the other two oil reference groups (Groups 4 and 5) had a mean intake of 14 g/day. The lower feed consumption in the oil reference groups compared to the soil and control feed groups is consistent with the expectation that these groups might consume less feed due to caloric intake from the oil gavage vehicle (9 kcal per g, or about 8 kcal per mL; USDA National Nutrient Database for Standard Reference, Release 17, 2004). This is approximately 15% of the caloric intake from feed observed in the soil groups, so the lower feed intake in the oil reference groups is consistent with an adjustment of feed intake by the animals, reflecting the caloric intake from corn-oil gavage.

The oil reference doses were prepared assuming that the rats in the soil group (Group 6) would consume 18 g/day, based on the pilot study results, so the observed daily feed intake matched what was anticipated. These intakes are somewhat lower than the 23 g/day that has been reported previously in the literature (Freeman et al. 1992).

### Body and Liver Weights

Rat body weights for all six dosing groups averaged 268 g at study initiation (study day -5), and 280 g at study termination (Figure 2; detailed data for all animals are presented in Table D-2), a gain of 4% over the 30-day study period. This weight gain reflects the fact that female Sprague-Dawley rats have already reached adult body weight at 4 months of age. Rat liver weights at study termination ranged from 8.1 to 12.2 g (average of 9.6 g) over all dosing groups, which is approximately 3.4% of body weight (Table D-3).

## Administered Doses

The average daily doses of compounds in each group are summarized in Table 6. As was intended, the administered dose was the highest for the soil group (Group 6), with a total mean TEQ dose of 2.1 ng/kg/day. The administered doses for the oil reference groups closely matched the proportional target doses, with mean TEQ doses that were 21%, 51%, and 83% of the dose to Group 6 for Groups 3, 4, and 5, respectively.

## PCDD/F Tissue Concentrations

Hepatic and adipose TEQ concentrations by dose group are summarized in Table 7. Concentrations of specific congeners of interest in liver and adipose tissues for each rat in the oil reference and soil dose groups are reported in Table D-4. Tissue concentrations of the congeners of interest were all above detection limits and were also greater than the instrument calibration limits in nearly all samples from the oil reference and soil groups. The concentrations of PCDD/F congeners in composited samples of hepatic and adipose tissue from the feed and oil control groups were uniformly low (Table D-5). The hepatic TEQ concentration of the soil group was intermediate between the concentrations attained in the 0.5X and 0.8X oil reference groups, and was statistically significantly different from both of these groups.

## EROD and MROD Activity

Mean EROD and MROD activities in rat liver tissue from all dose groups are reported in Table 8 and plotted in Figures 3 and 4, and the complete data set is presented in Tables D-6 and D-7. Both EROD and MROD displayed statistically significant increasing trends with increasing hepatic TEQ concentration, although the increase in MROD activity was much weaker than that seen for EROD activity (Figures 5 and 6). Mean MROD activities did not differ significantly among the oil reference groups and the soil group. However, there were statistically significant differences in mean EROD activity among the oil reference groups. The EROD activity in the soil group was statistically greater than that in the 0.2X and 0.5X oil reference groups (Groups 3 and 4), but was similar to that in the 0.8X oil reference group (Group 5).

## Fraction of Administered Dose Retained in Oil Reference Groups, by Congener

Figure 7 illustrates the fraction of administered dose present in liver and adipose tissues, and in the summed tissues, for all non-control dose groups. A larger proportion of administered dose was retained in liver than in adipose tissue for all dose groups for four of the five congeners of interest (Figures 7 and 8). For 2,3,7,8-TCDF, the fraction retained in adipose tissue was slightly higher in two dose groups (Groups 3 and 4), equal in the soil group (Group 6), and in one group, the fraction retained in liver was higher than the fraction retained in adipose tissue (Group 5).

The coefficient of variability among individual animals within each group was generally less than 15%.

The results of linear regressions across the three oil reference groups for fraction of administered dose retained (liver plus adipose burden) as a function of hepatic TEQ, EROD activity, and MROD activity are presented in Table 9 and illustrated in Figure 9. The fraction of TCDF retained was strongly and inversely related to hepatic EROD activity, with a weaker but statistically significant negative relationship to hepatic TEQ concentration. For three congeners—4-PeCDF, 1,2,3,4,7,8-HxCDF, and 1,2,3,6,7,8-HxCDF—positive relationships were observed between hepatic TEQ and fraction retained. No statistically significant relationship was observed between fraction of administered 1-PeCDF retained and either enzyme activity or hepatic TEQ concentration.

The results for TCDF are consistent with the hypothesis underlying this study, that the elimination rate for TCDF is dose-dependent due to induction of hepatic CYP1A1 activity with resulting increased elimination (and concomitant decreased retention) of this compound. The results for the three congeners that demonstrate positive relationships between hepatic TEQ and retained fraction of administered dose may be due to binding to induced CYP1A2 protein. 4-PeCDF and the higher chlorinated furans bind strongly to CYP1A2 protein (Diliberto et al. 1999). Although MROD activity was not statistically significantly different among most dose groups, it did demonstrate a statistically significant positive trend with increasing hepatic TEQ, indicating that some induction of CYP1A2 protein and activity was occurring. This protein induction may have been sufficient to increase the hepatic sequestration (and therefore the fraction of administered dose retained) of 4-PeCDF and the two HxCDF congeners with increasing dose among the oil reference groups.

## RBA Estimates

The results of the analysis of fraction retained as a function of hepatic TEQ and hepatic enzyme activity described above demonstrate that the elimination rates of four of the five tested congeners are affected by one or both of these parameters in the relevant dose range. Thus, the estimate of RBA obtained will vary depending on which oil reference group is used as the comparison (see Table D-8 for estimates of RBA based on each of the three oil reference groups). An accurate estimation of RBA for four of the five congeners requires comparing the retained fraction of administered dose between the soil group and an oil reference group matched on hepatic EROD activity and hepatic TEQ concentration. As discussed above, hepatic EROD activity in the soil group (Group 6) was similar to that in the 0.8X oil reference group (Group 5). Hepatic TEQ concentration in the soil group was intermediate between that observed in the 0.5X and 0.8X oil reference groups, and was statistically significantly different from both of these groups (see Table 7). Table 10 presents RBA calculations using both the 0.5X and 0.8X oil reference groups (Groups 4 and 5) as the basis for the calculations. While the two reference groups result in somewhat different estimates for individual congeners, the overall TEQ-weighted estimates of RBA are similar, regardless of which group is used.

Because the fractions of administered dose retained for four of the five tested congeners were significantly related to the hepatic TEQ concentration in the oil reference groups, the significant

differences between the soil and oil reference groups indicate that neither the 0.5X or the 0.8X groups (Groups 4 and 5) are accurate matches for the soil group. The dose-response relationships for fraction retained reported in Table 9 could be used to predict the fraction retained for each congener following administration in corn oil at the hepatic TEQ concentration observed in the soil group. These predicted values for fraction retained could then be used as the basis for a calculation of RBA at the matched hepatic TEQ concentration. However, given the close agreement between the RBA estimates obtained based on the 0.5X and 0.8X oil reference groups (60% vs. 58%, respectively), with estimates that fall well within the range of the CVs for the method, this additional step is probably unnecessary.

## Discussion

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The goals of this follow-up to the pilot bioavailability study were:

1. Evaluate EROD and MROD activity as a function of hepatic TEQ concentration in the tested dose range
2. Assess any dose-dependency of the elimination rate for each congener by examining the fraction of administered dose retained across dose rates
3. Base a revised RBA calculation on oil reference group(s) that match the soil group on hepatic TEQ and EROD activity, and compare the results to the original pilot-study results for rats and swine.

Observations regarding each of these goals based on results in the follow-up study are discussed below.

### Hepatic EROD/MROD Activities

Hepatic EROD and MROD activity both demonstrated a positive, statistically significant dose-response relationship among the three oil reference groups with increasing hepatic TEQ concentrations, but the trend was stronger for EROD activity, resulting in statistically significant differences in EROD activity among dose groups. The dose group differences in MROD activity were not significant among the three oil reference groups.

### Dose Dependence of Fraction Retained, by Congener

In this study, among the three oil reference groups with administered dose rates of 0.43, 1.1, and 1.7 ng TEQ/kg bodyweight per day, the fraction of administered dose retained at the end of 30 days was significantly affected by dose level for four of the five tested furan congeners. While the retained fraction of administered dose of TCDF decreased with increasing hepatic TEQ and EROD activity, the retained fractions of administered doses of 4-PeCDF, 1,2,3,4,7,8-HxCDF, and 1,2,3,6,7,8-HxCDF increased with increasing hepatic TEQ but were not statistically related to hepatic EROD activity. Thus, two different factors appear to be affecting the retention of administered dose:

1. For TCDF, previous studies suggested that CYP1A1 induction would enhance metabolism and therefore decrease retention. The results of this study are consistent with that hypothesis, and the fraction of administered TCDF retained at the end of 30 days was strongly dependent on hepatic EROD activity. For other congeners, there are also previous data suggesting elevated elimination rates at elevated dose rates, but in this study no relationship between hepatic EROD activity and fraction retained was observed for the other four tested congeners in the dose range evaluated.

2. For 4-PeCDF and the two HxCDF congeners tested, the observed increase in the fraction of administered dose retained with increasing hepatic TEQ may be due to induction of hepatic CYP1A2 protein. Although the trend in increasing MROD activity was relatively weak in the observed dose range, the increase in CYP1A2 protein may have been substantial enough to result in increased binding of these congeners to protein in the liver. This is supported by the slight trend of decreasing fraction retained in adipose tissue for these congeners (Figure 7), resulting in strong dose-related increases in the liver:adipose concentration ratio among the oil reference groups (Figure 8).

## Calculation of RBA and Comparisons with Pilot-Study Results

The results of the tests of trend in retained congener fractions indicate that the accuracy of any calculation of RBA for the soil congeners using the mass-balance method in this study depends on matches to two factors: hepatic EROD activity and hepatic TEQ concentration. As discussed above, the 0.8X oil reference group (Group 5) provided a good match to the soil group (Group 6) for hepatic EROD activity, while the hepatic TEQ concentration of the soil group was intermediate between the 0.5X and the 0.8X oil reference groups. Thus, the RBA calculation can be made using each of these two oil reference groups or, as discussed above, using the interpolated fractions of congeners retained between these groups at the mean hepatic TEQ concentration of the soil group.

The estimated RBAs obtained in this follow-up study can be assessed in comparison to the results from the pilot study. Figure 10 presents the RBA estimates for the tested floodplain congeners obtained in rats in both the pilot and follow-up studies. Several observations can be made based on these estimates:

- The RBA estimate for TCDF in rats was affected substantially when the reference group was matched on hepatic EROD activity or hepatic TEQ, as in the follow-up study. The estimates derived for TCDF in the follow-up study are now similar to the estimates obtained for the other four congeners tested, which ranged from 54% to 67%.
- The RBA estimates for rats for the remaining tested furan congeners were reasonably similar between the pilot and follow-up studies. Although the choice of reference group influenced the RBA estimates for three of the other (non-TCDF) congeners, the new estimates are generally within one standard deviation of the original estimate from the pilot study.

Figure 11 presents the estimated RBAs by congener based on rats in the follow-up study and based on swine from the pilot study. The RBA estimates obtained in the follow-up study for all tested congeners based on rats are still significantly different from those obtained using swine as the experimental model in the pilot study.

Table 11 presents the TEQ-weighted estimates of relative bioavailability for both species from the pilot study and from rats in the follow-up study, as well as estimates of absolute bioavailability calculated assuming that absolute oral bioavailability of all congeners in corn oil is 80%. This assumption is probably reasonable for the tetra- and penta- chlorinated congeners. However, experimental data on dioxin congeners suggest that more highly chlorinated congeners may have somewhat lower absolute bioavailability from corn oil, with octa-chlorinated congeners having very low absolute bioavailability from oil vehicles (less than 15%) (see data summarized in Table 1-1 of U.S. EPA 2003). The magnitude of change in the overall TEQ-weighted RBA estimate in rats for the floodplain soil sample is small. The pilot study yielded a TEQ-weighted RBA of 63% vs. 58–60% in the follow-up study.



## Conclusions and Recommendations

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### Conclusions

The follow-up study results demonstrate that:

- The elimination rates of four of the five furan congeners tested are dose-dependent, even in the relatively low-dose range tested here. Thus, any future studies of bioavailability conducted using the mass-balance approach relied on in this study should incorporate design features to ensure matching between soil and reference groups on hepatic TEQ concentration and EROD activity.
- Hepatic EROD induction itself cannot be used as a surrogate for estimating bioavailability. For the mixture of congeners tested here, hepatic EROD activity in the soil group was similar to that in the oil reference group given 80% of the same dose; however, on a mass-balance basis, the RBA was approximately 60% rather than 80%.
- The results of this follow-up study do not change the conclusion of the pilot study that, for the floodplain soil sample tested, the rat model results in statistically significantly higher estimated RBA than the swine model. This difference may be due to the mode of soil administration (soil mixed with feed in rats vs. soil samples wrapped in dough balls, with the dough balls prepared each day), or it may represent a true species difference in the gastrointestinal tract uptake of these compounds in soil. The soil/feed mixture used in the rat study was mixed thoroughly several weeks ahead of the 30-day study period. It is possible that prolonged contact between the soil and the relatively lipid-rich matrix of the feed could result in desorption of the contaminants into the feed, with resulting increase in apparent bioavailability from the soil. Alternatively, the observed species differences could represent true species differences in the extraction of dioxins and furans from the soil. Such differences are known for other types of compounds (for example, lead and other metals) (Weis and Lavelle 1991). Further experimentation and conclusions regarding the RBA of these compounds in humans should consider the comparative physiology of the rat and swine gastrointestinal tracts and the relative similarities and differences compared to human physiology (Karatli 1995; Miller and Ullrey 1987). However, a complete discussion of this issue is outside the scope of this report.

## Study Design Recommendations

If further bioavailability testing is conducted, several steps could be taken to refine the current study design somewhat and to reduce costs:

1. Costs could be reduced by compositing tissue samples from all individual animals within each oil reference group for HR-GC/MS. In both the pilot and the follow-up studies, the variability in fraction of administered dose retained among animals in each oil reference group was relatively low, with CVs in the range of 10%. Compositing tissues in the oil reference groups would reduce analytical costs substantially, and the baseline data here that indicate CVs of approximately 10% within oil reference groups could be carried forward in estimation of CVs for the RBA calculations. Quantitation of tissue concentrations in individual animals in tested soil groups could be retained.
2. Quantitation of hepatic CYP1A2 protein could be added to help match soil and oil reference groups on CYP1A2 induction. Protein determination is more sensitive than MROD activity for CYP1A2 protein induction, which appears to be related to hepatic sequestration (and increased retention) in the relevant dose ranges for some key congeners.
3. Fairly consistent RBA estimates across congeners were obtained when hepatic EROD activity and TEQ concentration are matched between the soil and oil reference groups. Given this, analytical costs could be reduced by selecting one congener for analysis and using this congener as a marker for overall bioavailability. Individual congeners that dominate the TEQ should be considered for selection. In floodplain soil samples, the two predominant congeners are 4-PeCDF (contributing approximately 50% of floodplain soil TEQ) and TCDF (approximately 25% of TEQ). The RBA estimates for TCDF appear to be more sensitive to experimental factors than those for 4-PeCDF. Given this, and the dominance of 4-PeCDF in the soil TEQ, 4-PeCDF could be used as a surrogate for the overall bioavailability of the furan contamination in the floodplain soils. Use of a single congener as the target for HR-GC/MS analysis would reduce analytical costs by more than 50%.

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## **Figures**

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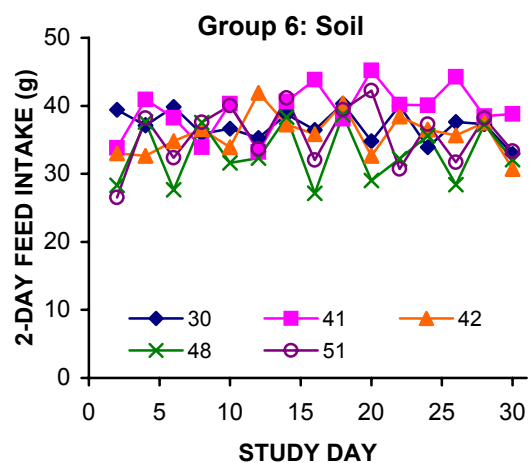
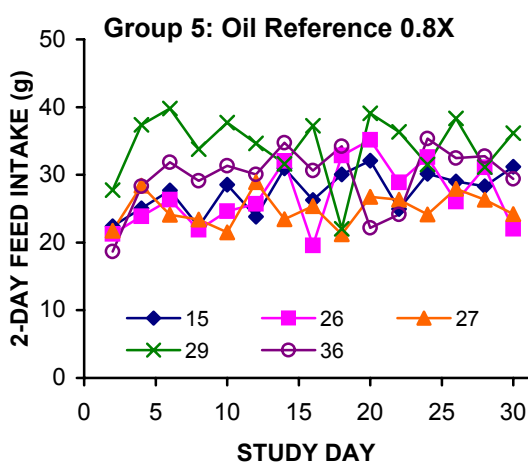
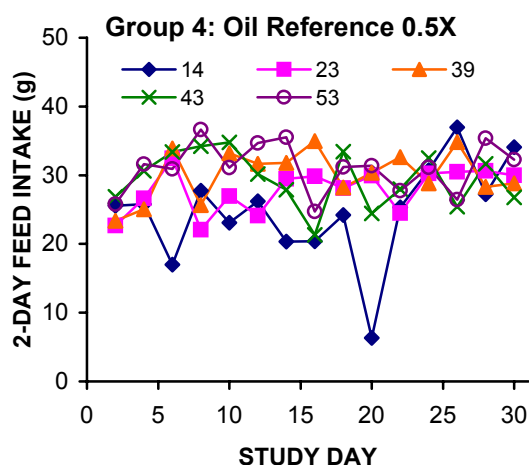
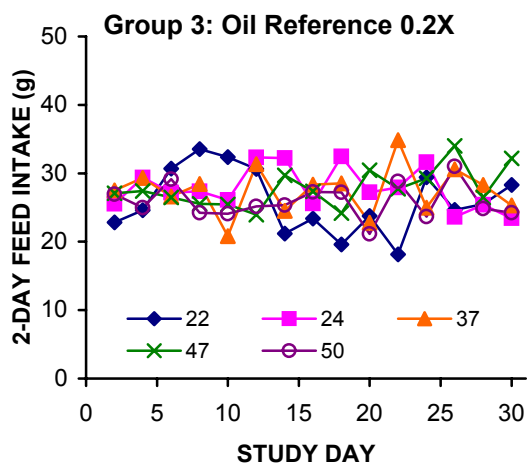
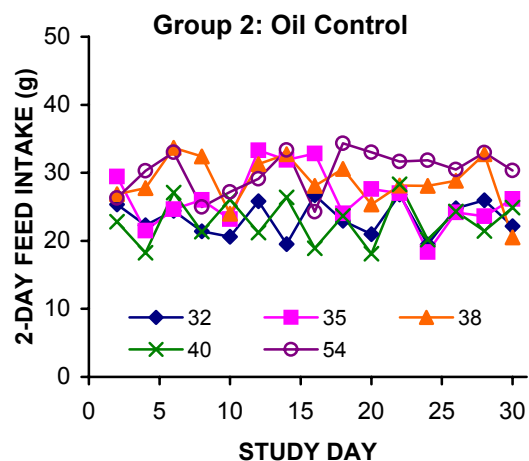
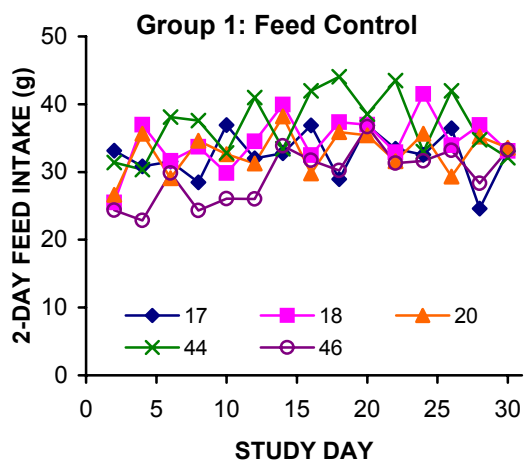


Figure 1. Feed intake for the follow-up rat study

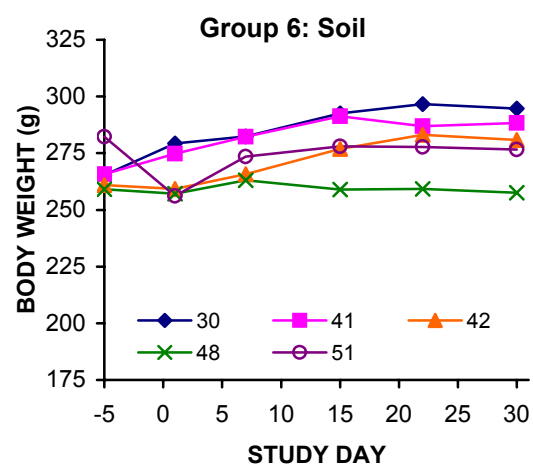
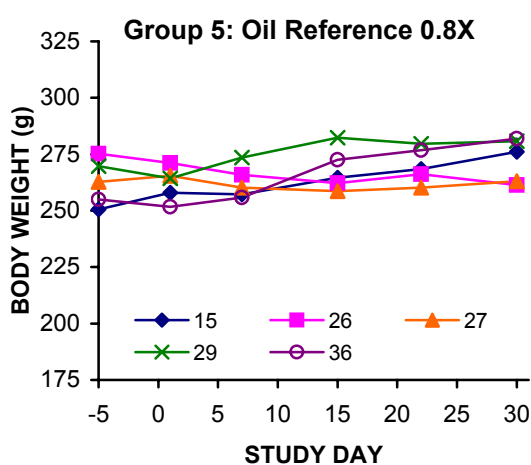
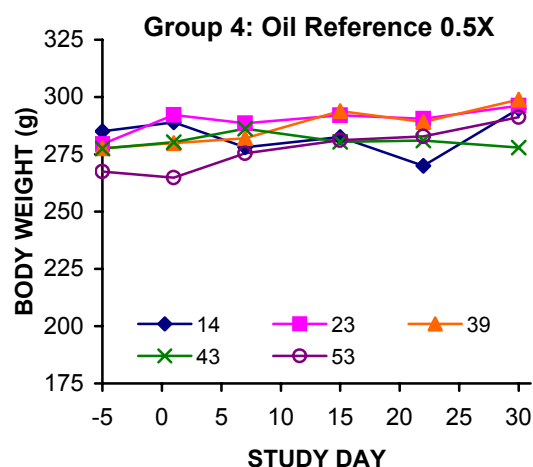
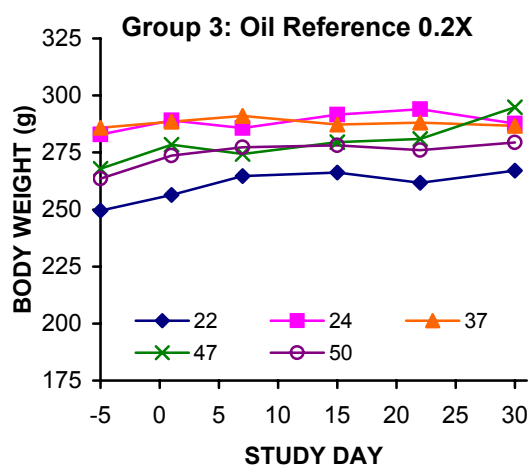
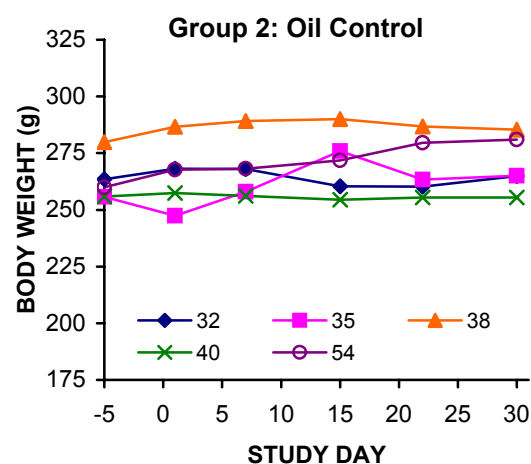
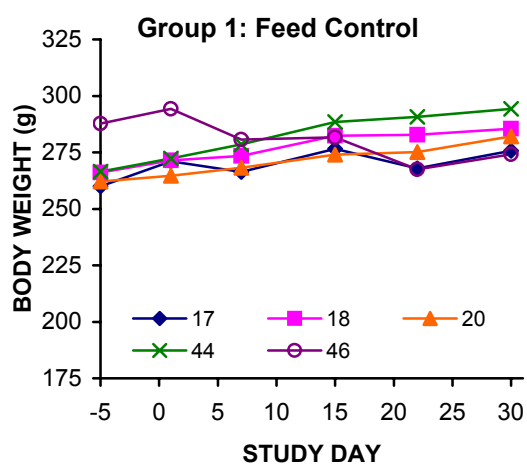
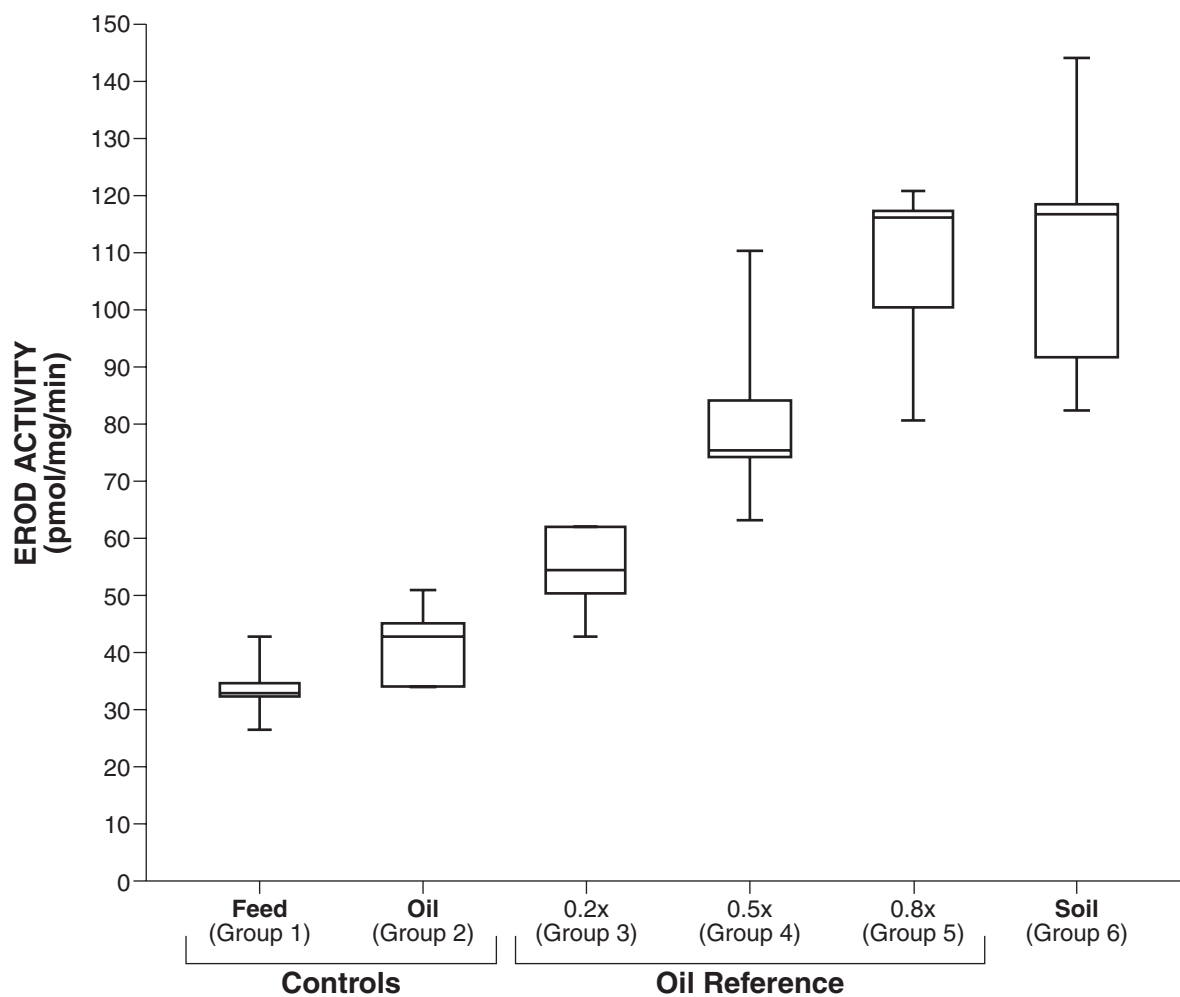
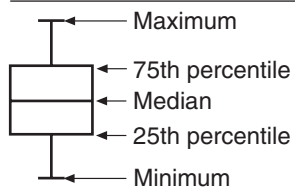


Figure 2. Body weights for the follow-up rat study



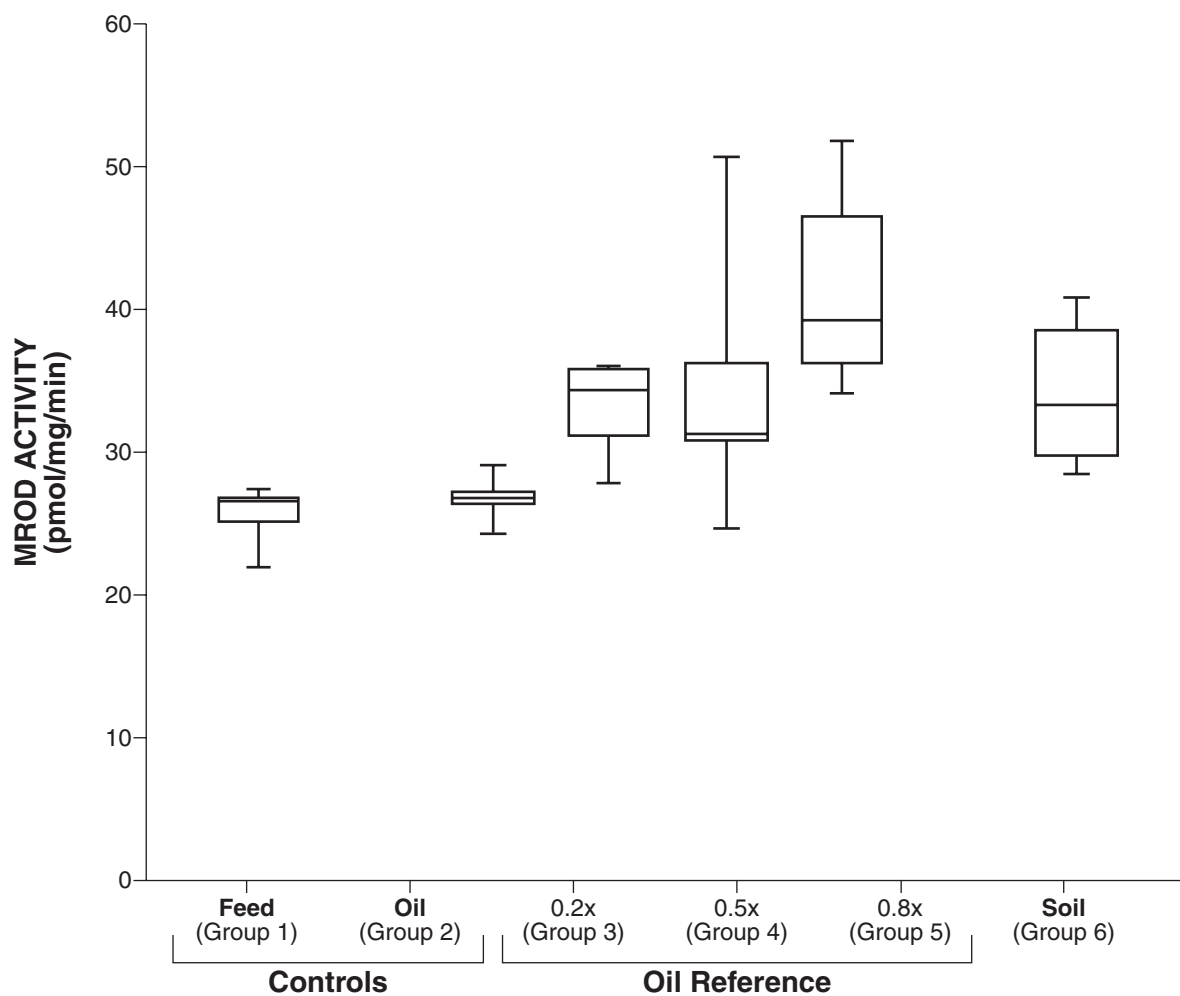
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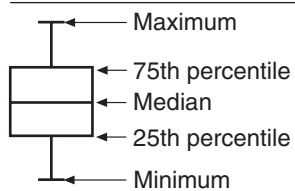
Note: The EROD activity in Group 6 was statistically elevated compared to all other groups except Group 5 (see Table 8).

Figure 3. EROD enzyme induction in the follow-up rat study





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Note: The only statistically significant difference among groups was between Groups 2 and 5 (see Table 8).

Figure 4. MROD enzyme induction in the follow-up rat study

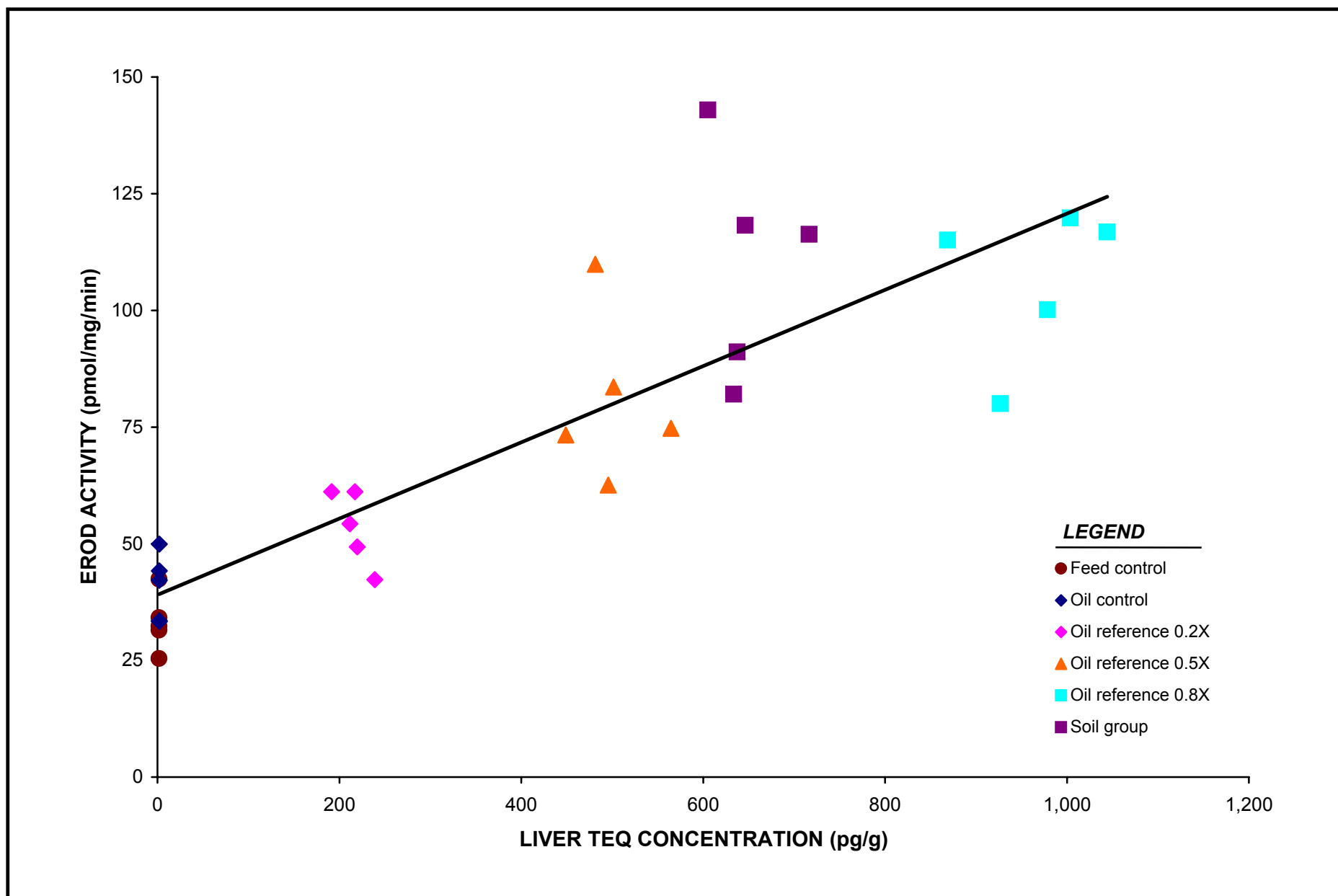


Figure 5. EROD activity vs. liver TEQ concentration in the rat follow-up study

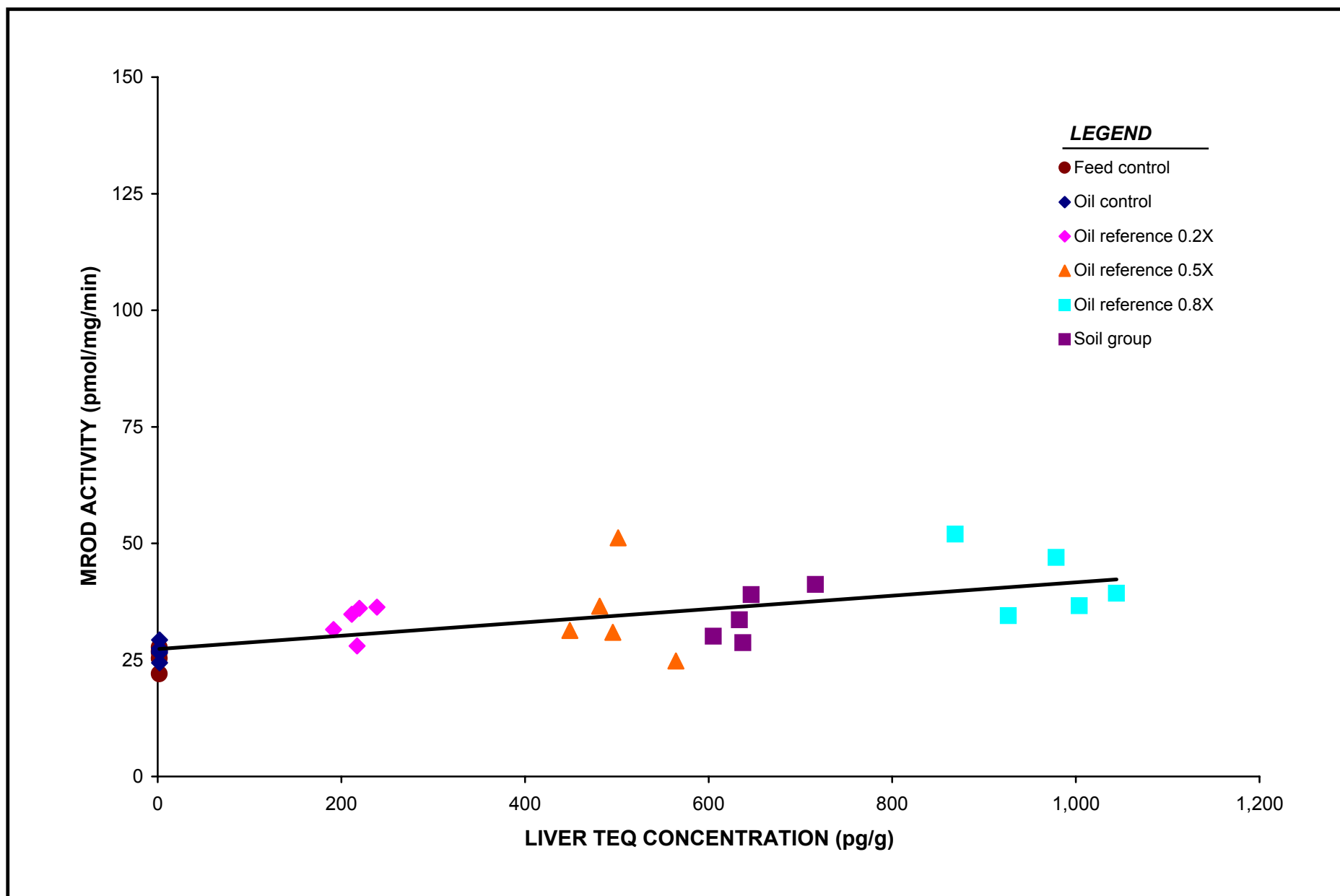


Figure 6. MROD activity vs. liver TEQ concentration in the rat follow-up study

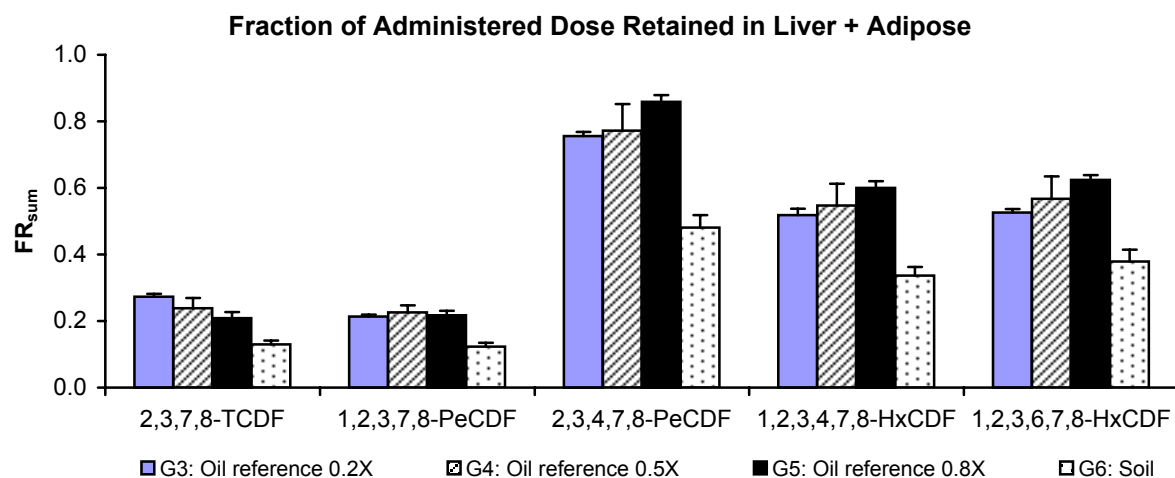
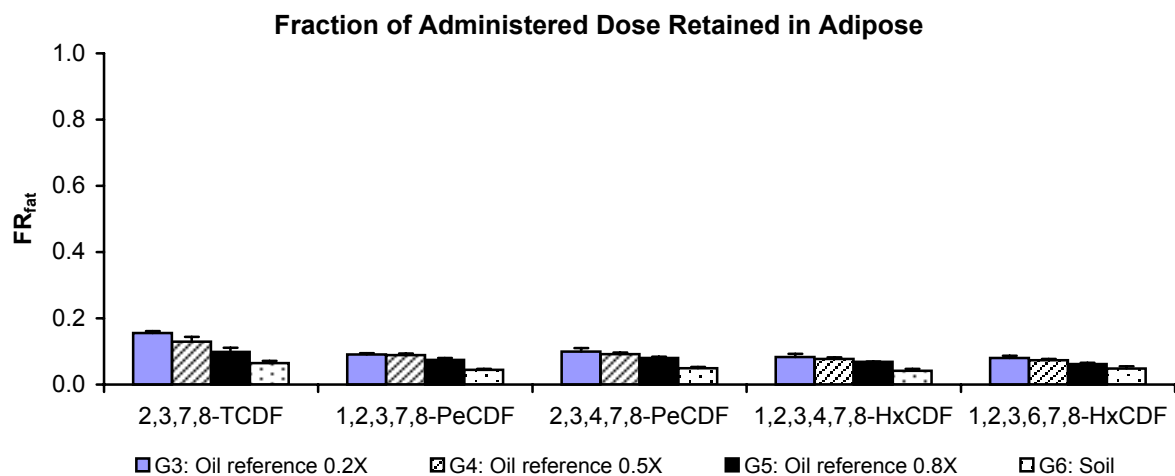
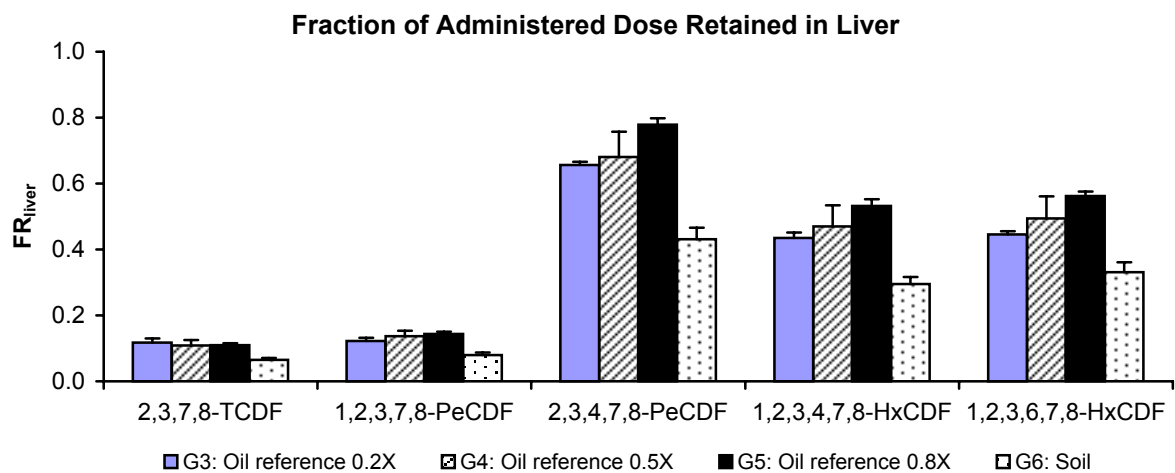


Figure 7. Distribution of administered doses in rat tissues

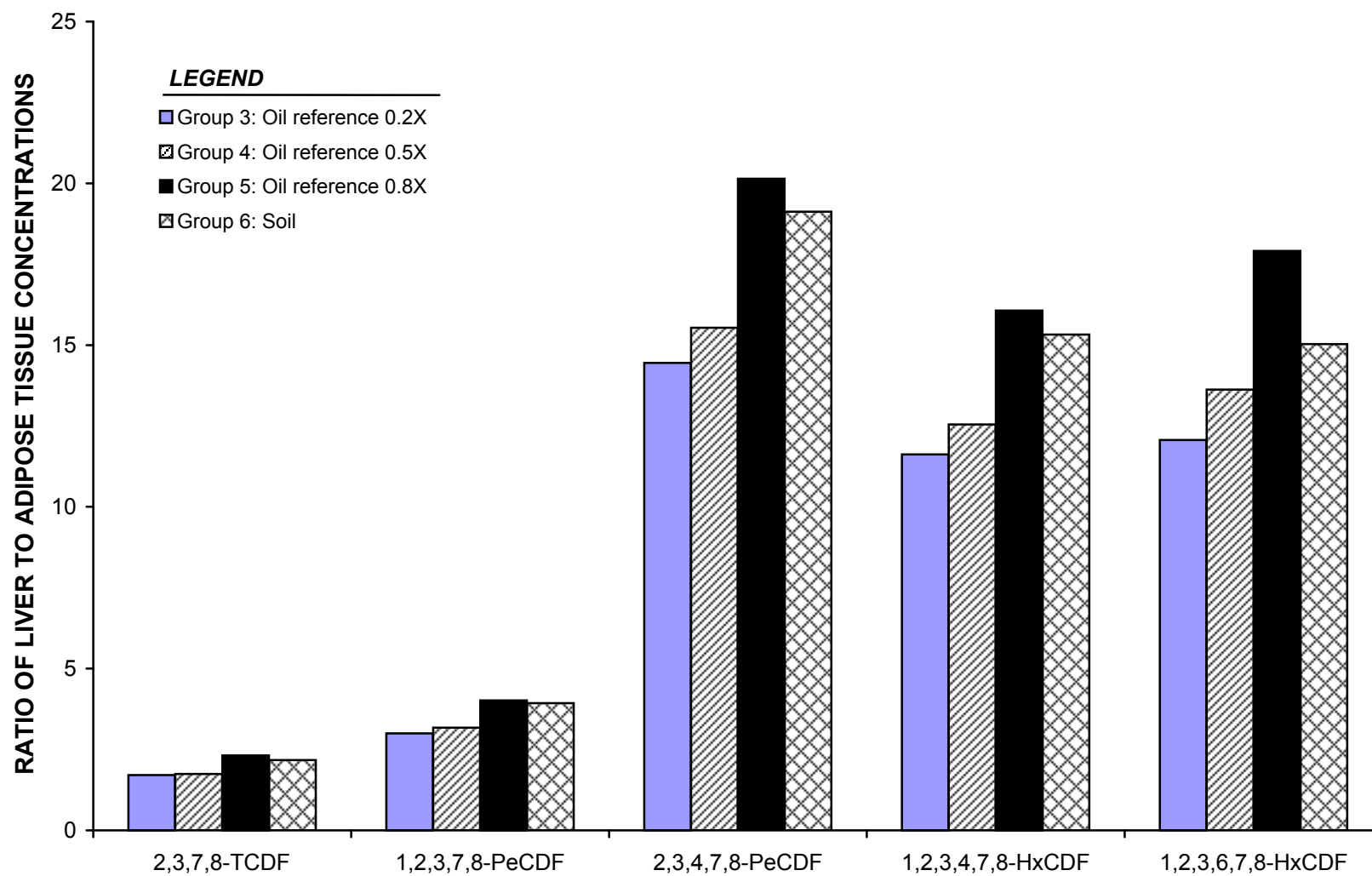


Figure 8. Ratio of liver to adipose tissue concentrations in the rat follow-up study

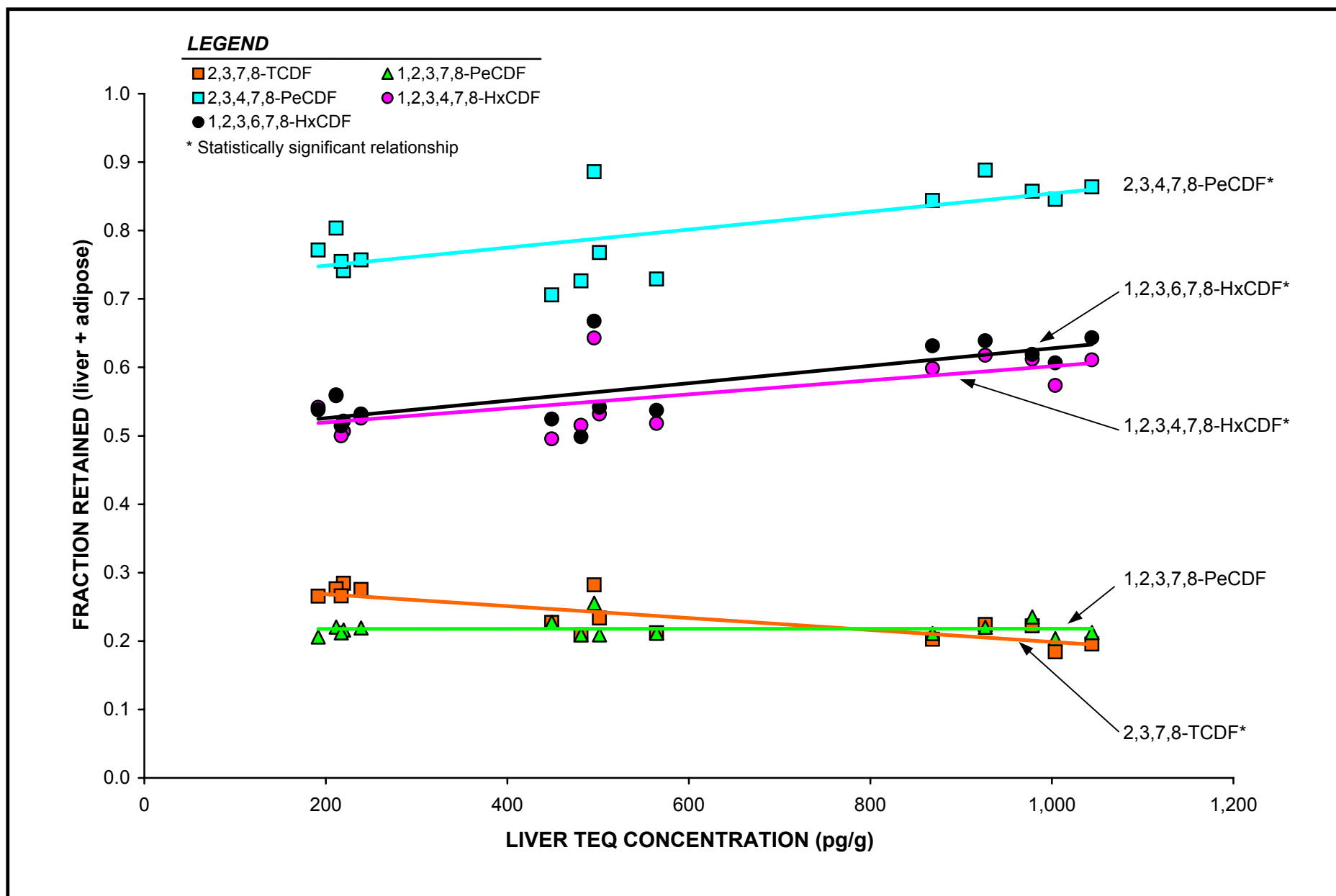


Figure 9. Fraction retained (sum of liver and adipose) vs. liver TEQ concentration in the rat follow-up study

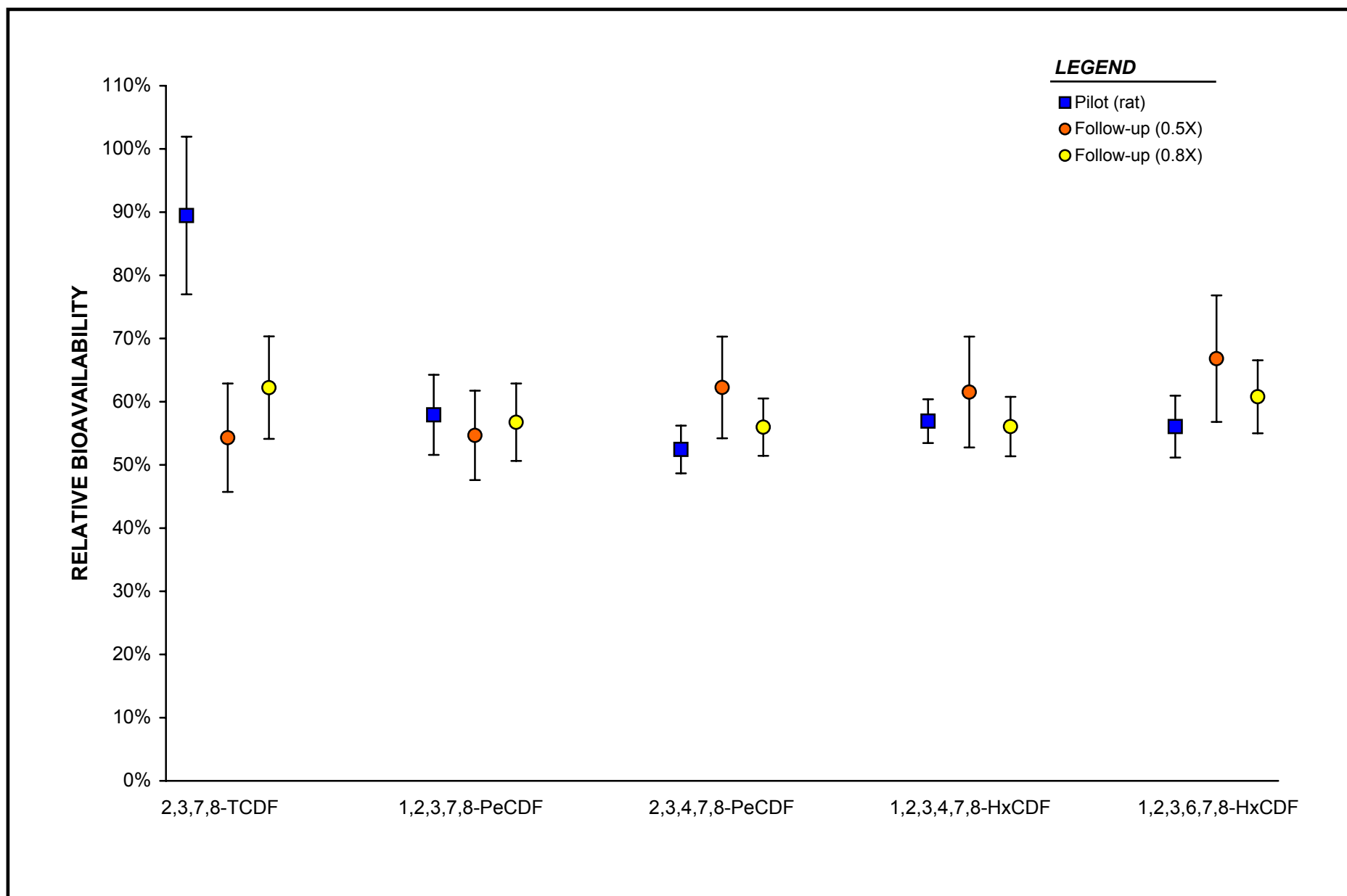


Figure 10. Comparison of RBAs (based on fraction retained in liver + adipose tissues) for rats between pilot and follow-up studies

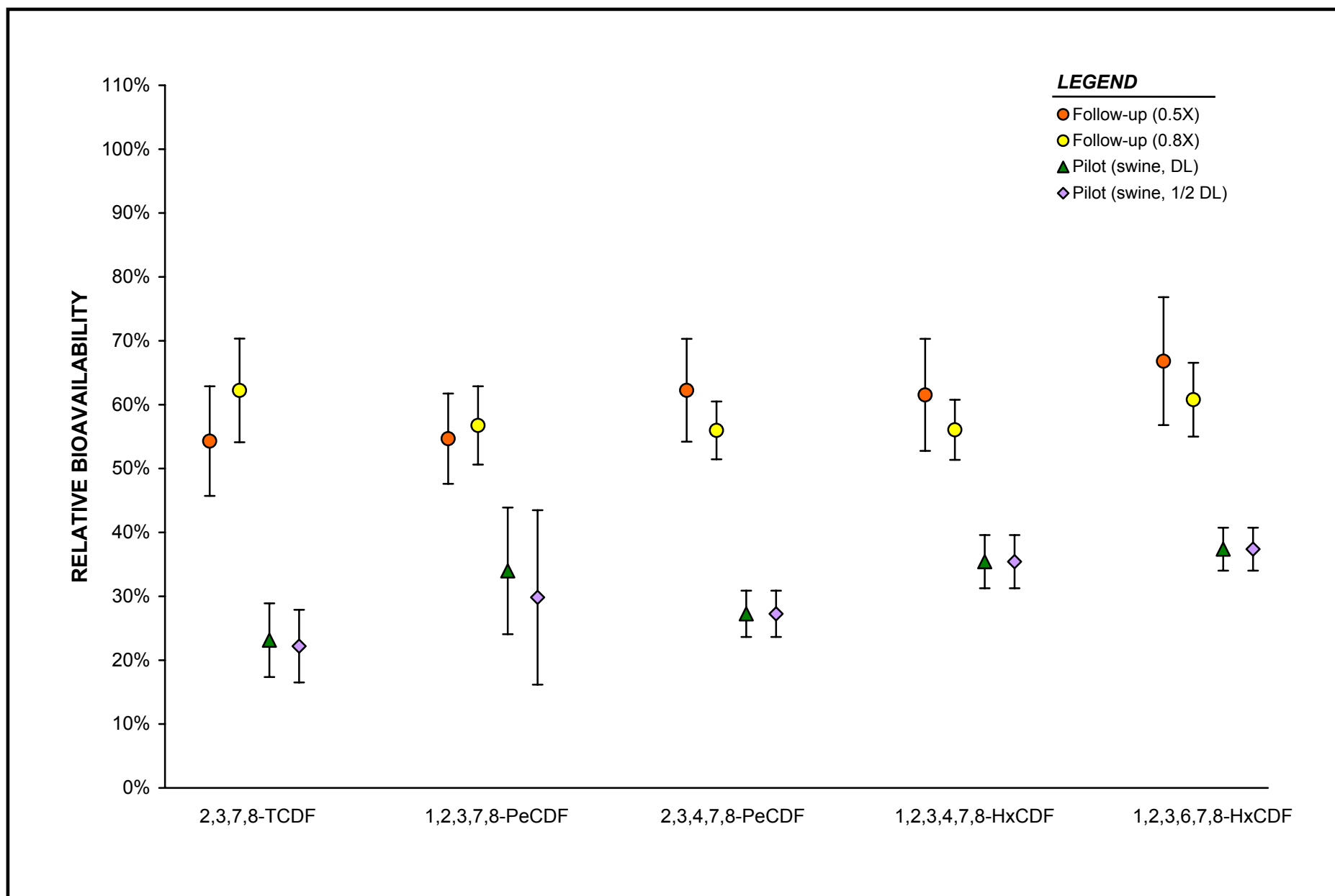


Figure 11. Comparison of RBAs (based on fraction retained in liver + adipose tissues) between swine (pilot study) and rats (follow-up study)



## **Tables**

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**Table 1. PCDD/F concentrations in triplicate samples of pilot study test soil (<250 µm)**

Sample Location:		Tittabawassee River Flood Plain Soil (Imerman Park 2)						
Sample ID:		THT02769						
Date:		7/8/2004						
Tag Number:		57273	57274	57275	Mean	Coefficient	TEQ	% of
Analyte	WHO TEF	Concentration (pg/g)	Concentration (pg/g)	Concentration (pg/g)	Concentration (pg/g)	of Variability (%)		
PCDDs/Fs								
2,3,7,8-TCDD	1	4.70	4.90	4.77	4.79	2.1%	4.79	0.6%
1,2,3,7,8-PeCDD	1	5.36 <i>J</i>	4.87	5.16	5.13	4.8%	5.13	0.6%
1,2,3,4,7,8-HxCDD	0.1	4.30 <i>J</i>	2.92 <i>U</i> <sup>a</sup>	3.60 <i>J</i>	3.61 <i>J</i>	19%	0.361	0.04%
1,2,3,6,7,8-HxCDD	0.1	26.3	18.7	17.9	21.0	22%	2.10	0.2%
1,2,3,7,8,9-HxCDD	0.1	8.04 <i>J</i>	7.30	7.68	7.67	4.8%	0.767	0.09%
1,2,3,4,6,7,8-HpCDD	0.01	490	383	346	406	18%	4.06	0.5%
OCDD	0.0001	4,540	3,820 <i>B</i>	3,530 <i>B</i>	3,963 <i>B</i>	13%	0.396	0.05%
2,3,7,8-TCDF	0.1	2,550 <i>E</i>	1,950	1,950	2,150	16%	215	25%
1,2,3,7,8-PeCDF	0.05	1,320	965	943	1,076	20%	53.8	6.3%
2,3,4,7,8-PeCDF	0.5	1,060	808	780	883	17%	441	52%
1,2,3,4,7,8-HxCDF	0.1	869	654	635	719	18%	71.9	8.5%
1,2,3,6,7,8-HxCDF	0.1	196 <i>D</i>	151 <i>D</i>	144 <i>D</i>	164 <i>D</i>	17%	16.4	1.9%
2,3,4,6,7,8-HxCDF	0.1	112	88.0	85.9	95.3	15%	9.53	1.1%
1,2,3,7,8,9-HxCDF	0.1	171	121	119	137	22%	13.7	1.6%
1,2,3,4,6,7,8-HpCDF	0.01	842	670	657 <i>D</i>	723	14%	7.23	0.9%
1,2,3,4,7,8,9-HpCDF	0.01	83.6	60.5	60.8	68.3	19%	0.683	0.08%
OCDF	0.0001	1,530	1,160	1,100	1,263	18%	0.126	0.01%
TEQ (pg/g)							847	
Other Parameters								
Solids, Total (%)	--	--	--	--	98.9	--	--	--
pH (s.u.)	--	--	--	--	7.69	--	--	--
Carbon, Total Organic (%)	--	--	--	--	2.73	--	--	--
Grain Size (%)								
Coarse sand (250 µm – 2 mm)	--	--	--	--	42.1	--	--	--
Fine sand (106 – 250 µm)	--	--	--	--	26.8	--	--	--
Very fine sand (75 – 106 µm)	--	--	--	--	8.78	--	--	--
Percent silt (4 – 75 µm)	--	--	--	--	21.4	--	--	--
Percent clay (< 4 µm)	--	--	--	--	0.86	--	--	--

**Note:** These results are the same as those presented in the pilot study report. The soil sample was not re-analyzed for the follow-up study.

*B* – This compound was also detected in the method blank.

*D* – The amount reported is the maximum possible concentration due to possible chlorinated diphenylether interference.

*E* – The amount detected is above the Upper Calibration Limit of the instrument.

*J* – The amount detected is below the Lower Calibration Limit of the instrument.

*U* – Not detected; value represents the sample-specific detection limit, unless noted otherwise.

TEQ – Toxicity Equivalence Concentration

WHO TEF – World Health Organization Toxicity Equivalence Factor

Highlighting indicates the five congeners that contribute most to the total TEQ

If more than half of the results for a chemical were qualified with a *B*, *D*, *E*, or *J*, then the associated mean concentration was also qualified.

<sup>a</sup> Nondetect reported to the EMPC (Estimated Maximum Possible Concentration).

**Table 2. PCDD/F concentrations in Rodent Lab Diet 5001 and corn oil**

Analyte	Sample ID: Date:		Rodent Lab Diet 5001 2/24/2006		Corn Oil (Spectrum Chemical) 2/24/2006	
	WHO TEF		Concentration (pg/g)	TEQ (pg/g)	Concentration (pg/mL)	TEQ (pg/mL)
<b>PCDDs/Fs</b>						
2,3,7,8-TCDD	1		0.0852 <i>U</i>	0.0852	0.599 <i>U</i>	0.599
1,2,3,7,8-PeCDD	1		0.0756 <i>U</i>	0.0756	0.569 <i>U</i>	0.569
1,2,3,4,7,8-HxCDD	0.1		0.0815 <i>U</i>	0.00815	1.07 <i>U</i>	0.107
1,2,3,6,7,8-HxCDD	0.1		0.0833 <i>U</i>	0.00833	1.03 <i>U</i>	0.103
1,2,3,7,8,9-HxCDD	0.1		0.0745 <i>U</i> <sup>a</sup>	0.00745	0.990 <i>U</i>	0.0990
1,2,3,4,6,7,8-HpCDD	0.01		0.850 <i>J</i>	0.00850	0.816 <i>U</i>	0.00816
OCDD	0.0001		10.2 <i>B</i>	0.00102	6.50 <i>J</i>	0.00065
2,3,7,8-TCDF	0.1		0.157 <i>J</i>	0.0157	0.834 <i>U</i>	0.0834
1,2,3,7,8-PeCDF	0.05		0.0861 <i>U</i>	0.00431	1.01 <i>U</i>	0.0505
2,3,4,7,8-PeCDF	0.5		0.0546 <i>U</i> <sup>a</sup>	0.0273	0.959 <i>U</i>	0.480
1,2,3,4,7,8-HxCDF	0.1		0.0281 <i>U</i>	0.00281	0.282 <i>U</i>	0.0282
1,2,3,6,7,8-HxCDF	0.1		0.0264 <i>U</i>	0.00264	0.254 <i>U</i>	0.0254
2,3,4,6,7,8-HxCDF	0.1		0.0290 <i>U</i>	0.00290	0.286 <i>U</i>	0.0286
1,2,3,7,8,9-HxCDF	0.1		0.0451 <i>U</i>	0.00451	0.436 <i>U</i>	0.0436
1,2,3,4,6,7,8-HpCDF	0.01		0.110 <i>U</i>	0.00110	0.400 <i>U</i>	0.00400
1,2,3,4,7,8,9-HpCDF	0.01		0.138 <i>U</i>	0.00138	0.460 <i>U</i>	0.00460
OCDF	0.0001		0.335 <i>J</i>	3.35E-05	2.25 <i>U</i>	0.000225
<b>TEQ</b>				<b>0.257</b>		<b>2.234</b>

**Note:** *J* – The amount detected is below the Lower Calibration Limit of the instrument.

*U* – Not detected; value represents the sample-specific detection limit, unless noted otherwise.

TEQ – Toxicity Equivalence Concentration

WHO TEF – World Health Organization Toxicity Equivalence Factor

<sup>a</sup> Nondetect reported to the EMPC (Estimated Maximum Possible Concentration).

**Table 3. PCDD/F concentrations in blended rat diet**

Sample ID: Date:		Soil THT02769/Diet Blend 11/16/2005											
Analyte	WHO TEF	Pre-Dosing Analysis							Post-Dosing Analysis (pg/g)	Pre- and Post-Dosing Analysis			
		Top (#1) Concentration (pg/g)	Middle (#2) Concentration (pg/g)	Bottom (#3) Concentration (pg/g)	Mean Concentration (pg/g)	Standard Deviation (pg/g)	Coefficient of Variability (%)	Mean Concentration (pg/g)		Coefficient of Variability (%)	TEQ (pg/g)	% of TEQ	
2,3,7,8-TCDD	1	0.369 <i>U</i>	0.344 <i>U</i>	0.480 <i>J</i>	0.398 <i>U</i>	0.072	18%	0.311 <i>J</i>	0.354 <i>J</i>	19%	0.354	0.9%	
1,2,3,7,8-PeCDD	1	0.407 <i>U</i>	0.384 <i>U</i>	0.487 <i>U</i>	0.426 <i>U</i>	0.054	13%	0.357 <i>U</i> <sup>a</sup>	0.392 <i>U</i>	14%	0.392	1.0%	
1,2,3,4,7,8-HxCDD	0.1	0.593 <i>U</i>	0.532 <i>U</i>	0.640 <i>U</i>	0.588 <i>U</i>	0.054	9.2%	0.262 <i>U</i> <sup>a</sup>	0.425 <i>U</i>	33%	0.0425	0.1%	
1,2,3,6,7,8-HxCDD	0.1	1.75 <i>J</i>	1.28 <i>U</i> <sup>a</sup>	1.54 <i>J</i>	1.52 <i>J</i>	0.24	15%	2.17 <i>J</i>	1.85 <i>J</i>	22%	0.185	0.5%	
1,2,3,7,8,9-HxCDD	0.1	0.601 <i>U</i>	0.494 <i>U</i>	0.585 <i>U</i>	0.560 <i>U</i>	0.058	10%	0.724 <i>J</i>	0.642 <i>U</i>	16%	0.0642	0.2%	
1,2,3,4,6,7,8-HpCDD	0.01	29.8	27.4	26.1	27.8	1.9	6.8%	31.7	29.7	8.7%	0.297	0.8%	
OCDD	0.0001	257	220	204	227	27	12%	237 <i>B</i>	232	9.9%	0.0232	0.1%	
2,3,7,8-TCDF	0.1	67.1	67.7	75.5	70.1	4.7	6.7%	88.4	79.3	13%	7.93	21%	
1,2,3,7,8-PeCDF	0.05	46.4	48.7	54.0	49.7	3.9	7.8%	49.2	49.5	6.4%	2.48	6.4%	
2,3,4,7,8-PeCDF	0.5	38.6	39.7	44.3	40.9	3.0	7.4%	43.7	42.3	6.8%	21.2	55%	
1,2,3,4,7,8-HxCDF	0.1	31.3	34.3	38.8	34.8	3.8	11%	32.0	33.4	9.9%	3.34	8.7%	
1,2,3,6,7,8-HxCDF	0.1	8.41	7.71	8.93	8.35	0.61	7.3%	8.02	8.19	6.4%	0.819	2.1%	
2,3,4,6,7,8-HxCDF	0.1	4.17	4.25	4.19	4.20	0.042	1.0%	4.11 <i>J</i>	4.16	1.4%	0.416	1.1%	
1,2,3,7,8,9-HxCDF	0.1	6.38	6.60	7.41	6.80	0.54	8.0%	6.48	6.64	7.0%	0.664	1.7%	
1,2,3,4,6,7,8-HpCDF	0.01	33.3	32.7	32.7	32.9	0.35	1.1%	38.6	35.8	8.3%	0.358	0.9%	
1,2,3,4,7,8,9-HpCDF	0.01	2.98	3.67	3.69	3.45	0.40	12%	3.20 <i>J</i>	3.32	10%	0.0332	0.1%	
OCDF	0.0001	59.1	60.7	55.7	58.5	2.6	4.4%	68.5	63.5	8.9%	0.00635	0.02%	

**Note:** *J* – The amount detected is below the Lower Calibration Limit of the instrument.

*U* – Not detected; value represents the sample-specific detection limit, unless noted otherwise.

TEQ – Toxicity Equivalence Concentration

WHO TEF – World Health Organization Toxicity Equivalence Factor

Highlighting indicates the five congeners in each sample that contribute most to the total TEQ.

If more than half of the results for a chemical were qualified with a *U* or *J*, then the associated mean concentration was also qualified.

<sup>a</sup> Nondetect reported to the EMPC (Estimated Maximum Possible Concentration).

**Table 4. Analytical results for oil reference mixtures used in follow-up rat study**

Analyte	Target Concentration (pg/mL)	Pre-Dosing Measured Concentration (pg/mL)	Relative Percent Difference <sup>a</sup>	Post-Dosing Measured Concentration (pg/mL)	Average Measured Concentration <sup>b</sup> (pg/mL)	Coefficient of Variability <sup>c</sup>
<b>Group 3: Oil Reference 0.2X</b>						
2,3,7,8-TCDF	252	267	5.6%	268	268	0.3%
1,2,3,7,8-PeCDF	179	188	4.9%	182	185	2.3%
2,3,4,7,8-PeCDF	147	161	8.9%	171	166	4.3%
1,2,3,4,7,8-HxCDF	125	121	3.5%	123	122	1.2%
1,2,3,6,7,8-HxCDF	30.1	34.7	14%	37.2	36.0	4.9%
<b>Group 4: Oil Reference 0.5X</b>						
2,3,7,8-TCDF	631	645	2.2%	700	673	5.8%
1,2,3,7,8-PeCDF	447	439	1.9%	465	452	4.1%
2,3,4,7,8-PeCDF	368	385	4.5%	459	422	12%
1,2,3,4,7,8-HxCDF	313	291	7.3%	322	307	7.2%
1,2,3,6,7,8-HxCDF	75.2	78.4	4.2%	100	89.2	17%
<b>Group 5: Oil Reference 0.8X</b>						
2,3,7,8-TCDF	1,009	976	3.4%	1,070	1,023	6.5%
1,2,3,7,8-PeCDF	716	690	3.7%	724	707	3.4%
2,3,4,7,8-PeCDF	589	594	0.9%	689	642	10%
1,2,3,4,7,8-HxCDF	501	450	11%	488	469	5.7%
1,2,3,6,7,8-HxCDF	120	127	5.5%	145	136	9.4%

<sup>a</sup> The relative percent difference (RPD) between the target and pre-dosing measured concentrations is calculated as the absolute value of the difference divided by the average of the target and pre-dosing measured concentrations.

<sup>b</sup> Average of pre- and post-dosing measured concentrations.

<sup>c</sup> Coefficient of variability between pre- and post-dosing measured concentrations.

**Table 5. Dose groups and test materials used in the rat follow-up study**

Dose Group	Group Name	Description
1	Feed control	Undosed control group, fed clean feed, no gavage
2	Oil control	Undosed control group, fed clean feed, gavaged with unspiked corn oil
3	Oil reference 0.2X	Reference group, with corn oil spiked at 20% of calculated PCDD/F dose administered to Group 6
4	Oil reference 0.5X	Reference group, with corn oil spiked at 50% of calculated PCDD/F dose administered to Group 6
5	Oil reference 0.8X	Reference group, with corn oil spiked at 80% of calculated PCDD/F dose administered to Group 6
6	Soil group	Tittabawassee River floodplain soil blended with diet, nominal daily dose rate X

**Table 6. Average daily doses administered to rats**

	WHO TEF	Soil (Group 6)			Oil Reference 0.2X (Group 3)			Oil Reference 0.5X (Group 4)			Oil Reference 0.8X (Group 5)		
		Average Daily Dose (ng/kg bw/day)			Average Daily Dose (ng/kg bw/day)			Average Daily Dose (ng/kg bw/day)			Average Daily Dose (ng/kg bw/day)		
		Mean	SD	TEQ	Mean	SD	TEQ	Mean	SD	TEQ	Mean	SD	TEQ
2,3,7,8-TCDF	0.1	5.20	0.17	0.520	0.959	0.038	0.0959	2.36	0.044	0.236	3.83	0.0776	0.383
1,2,3,7,8-PeCDF	0.05	3.24	0.11	0.162	0.662	0.026	0.0331	1.59	0.030	0.0794	2.65	0.0536	0.132
2,3,4,7,8-PeCDF	0.5	2.77	0.091	1.39	0.594	0.023	0.297	1.48	0.028	0.741	2.40	0.0487	1.20
1,2,3,4,7,8-HxCDF	0.01	2.19	0.072	0.0219	0.436	0.017	0.00436	1.08	0.020	0.0108	1.76	0.0356	0.0176
1,2,3,6,7,8-HxCDF	0.01	0.537	0.018	0.00537	0.129	0.0050	0.00129	0.313	0.00588	0.00313	0.509	0.0103	0.00509
<b>Total Mean TEQ Dose:</b>		--	--	<b>2.10</b>	--	--	<b>0.431</b>	--	--	<b>1.07</b>	--	--	<b>1.74</b>

**Notes:**

All dose groups used for analyses were comprised of 5 animals

WHO TEF – World Health Organization Toxicity Equivalence Factor

SD – Standard deviation

TEQ – Toxicity Equivalence Concentration

**Table 7. Summary of TEQ concentrations in liver and adipose tissues**

Group/Tissue	TEQ Concentrations (pg/g)		Statistical Analysis <sup>a</sup>
	Average	SD	
<b>Group 1: Feed Control</b>			
Liver	0.719 <sup>b</sup>	--	--
Fat	0.199 <sup>b</sup>	--	--
<b>Group 2: Oil Control</b>			
Liver	0.877 <sup>b</sup>	--	--
Fat	0.210 <sup>b</sup>	--	--
<b>Group 3: Oil Reference (0.2X)</b>			
Liver	216	17	Significantly different from Group 6
Fat	21.6	1.3	Significantly different from Group 6
<b>Group 4: Oil Reference (0.5X)</b>			
Liver	498	42	Significantly different from Group 6
Fat	45.5	3.3	Not significantly different from Group 6
<b>Group 5: Oil Reference (0.8X)</b>			
Liver	964	68	Significantly different from Group 6
Fat	65.9	3.0	Significantly different from Group 6
<b>Group 6: Soil</b>			
Liver	648	41	Significantly different from all other groups
Fat	49.4	2.2	Significantly different from all other groups

<sup>a</sup> Comparisons were conducted using an ANOVA followed by Dunnett's multiple comparison test at an overall 95 percent confidence level (overall alpha = 0.05).

<sup>b</sup> Laboratory analyses were performed on a composite sample of all five rats in group.



**Table 8. Summary of EROD and MROD liver microsomal activity data**

	N	Liver Microsomal Activities (pmol/mg/min)				Conclusion
		Minimum	Maximum	Mean	SD	
<b>EROD</b>						
G1: Feed control	5	25.4	42.4	33.2	6.1	not significantly different from G2 <sup>a</sup>
G2: Oil control	5	33.4	49.9	40.6	7.2	significantly lower than G4 and G5 <sup>b</sup>
G3: Oil reference 0.2x	5	42.3	61.2	53.6	8.1	not significantly different from G2 <sup>b</sup>
G4: Oil reference 0.5x	5	62.6	109.9	80.8	17.9	significantly higher than G2 <sup>b</sup>
G5: Oil reference 0.8x	5	80.0	119.8	106.4	16.6	significantly higher than G2 <sup>b</sup>
G6: Soil	5	82.0	142.9	110.1	24.1	significantly higher than all groups except G5 <sup>b</sup>
<b>MROD</b>						
G1: Feed control	5	22.0	27.7	25.7	2.2	not significantly different from G2 <sup>a</sup>
G2: Oil control	5	24.4	29.3	26.9	1.8	significantly lower than G5 <sup>b</sup>
G3: Oil reference 0.2x	5	28.0	36.3	33.3	3.6	not significantly different from G2 <sup>b</sup>
G4: Oil reference 0.5x	5	24.8	51.2	34.9	10.0	not significantly different from G2 <sup>b</sup>
G5: Oil reference 0.8x	5	34.5	52.0	41.9	7.4	significantly higher than G2 <sup>b</sup>
G6: Soil	5	28.7	41.2	34.5	5.5	not significantly different from any <sup>b</sup>

**Notes:** EROD – ethoxyresorufin O-deethylase  
MROD – methoxyresorufin O-deethylase  
SD – standard deviation

<sup>a</sup> Groups G1 and G2 compared using standard t-tests; Comparisons using Wilcoxon non-parametric test provided identical conclusions.

<sup>b</sup> Comparisons with groups G2 and G6 were each conducted using an ANOVA followed by Dunnett's multiple comparison test at an overall 95 percent confidence level (overall alpha = 0.05)

**Table 9. Statistical analysis of fraction of administered dose retained vs. hepatic TEQ, EROD activity, and MROD activity**

	Regression Coefficients									
	TCDF		1-PeCDF		4-PeCDF		1,2,3,4,7,8-HxCDF		1,2,3,6,7,8-HxCDF	
	$\beta$	p	$\beta$	p	$\beta$	p	$\beta$	p	$\beta$	p
Intercept	0.31	<0.0001	0.24	<0.0001	0.76	<0.0001	0.52	<0.0001	0.54	<0.0001
Hepatic TEQ (pg/g)	-1.9E-05	NS	3.3E-05	NS	0.00023	<0.01	0.00017	<0.01	0.00022	<0.01
EROD (pmol/mg/min)	-0.0011	<0.01	-0.000491	NS	-0.0016	NS	-0.0012	NS	-0.0015	NS
MROD (pmol/mg/min)	0.00077	NS	3.3E-05	NS	0.0011	NS	0.00089	NS	0.00063	NS
p for model <sup>b</sup>	<0.0001		NS		<0.05		<0.05		<0.01	

**Note:** NS – not significant

<sup>a</sup> Multivariate linear regression (least squares method)

<sup>b</sup> F-test significance

**Table 10. Relative bioavailability estimates for the follow-up rat study based on 0.5X and 0.8X reference oil groups**

Congener	Percent of Soil TEQ	Fraction Retained (liver + adipose)						Relative Bioavailability			
		Soil (Group 6)		0.5X (Group 4)		0.8X (Group 5)		Using 0.5X (Group 4)		Using 0.8X (Group 5)	
		Mean	SD	Mean	SD	Mean	SD	Mean	CV	Mean	CV
2,3,7,8-TCDF	25.4%	0.13	0.012	0.24	0.030	0.21	0.019	54%	16%	62%	13%
1,2,3,7,8-PeCDF	6.3%	0.12	0.011	0.23	0.021	0.22	0.014	55%	13%	57%	11%
2,3,4,7,8-PeCDF	52.1%	0.48	0.037	0.77	0.080	0.86	0.021	62%	13%	56%	8.1%
1,2,3,4,7,8-HxCDF	8.5%	0.34	0.026	0.55	0.066	0.60	0.020	62%	14%	56%	8.4%
1,2,3,6,7,8-HxCDF	1.9%	0.38	0.035	0.57	0.067	0.62	0.014	67%	15%	61%	10%
<b>TEQ-Weighted:</b>								<b>60%</b>		<b>58%</b>	

**Notes:** RBA – relative bioavailability, calculated using Equation 1 (see text)

SD – standard deviation

CV – coefficient of variability  $CV = (CV_{\text{soil}}^2 + CV_{\text{reference}}^2)^{0.5}$

**Table 11. TEQ-weighted relative and absolute bioavailability estimates for the pilot and follow-up studies**

Congener	Percent of Soil TEQ	Mean RBA <sup>a</sup>					Estimated Absolute Bioavailability <sup>b</sup>				
		Pilot			Follow-Up, Rat		Pilot			Follow-Up, Rat	
		Rat	Swine				Rat	Swine			
			ND=1/2 DL	ND=DL	Using 0.5X <sup>c</sup>	Using 0.8X <sup>d</sup>		ND=1/2 DL	ND=DL	Using 0.5X <sup>c</sup>	Using 0.8X <sup>d</sup>
Tittabawassee River Flood Plain Soil											
2,3,7,8-TCDF	25.4%	0.89	0.22	0.23	0.54	0.62	0.72	0.18	0.18	0.43	0.50
1,2,3,7,8-PeCDF	6.3%	0.58	0.30	0.34	0.55	0.57	0.46	0.24	0.27	0.44	0.45
2,3,4,7,8-PeCDF	52.1%	0.52	0.27	0.27	0.62	0.56	0.42	0.22	0.22	0.50	0.45
1,2,3,4,7,8-HxCDF	8.5%	0.57	0.35	0.35	0.62	0.56	0.46	0.28	0.28	0.49	0.45
1,2,3,6,7,8-HxCDF	1.9%	0.56 <sup>e</sup>	0.37	0.37	0.67	0.61	0.45 <sup>e</sup>	0.30	0.30	0.53	0.49
TEQ-Weighted:		0.63	0.27	0.27	0.60	0.58	0.51	0.22	0.22	0.48	0.46

<sup>a</sup> RBA estimates for soil compared to corn oil reference material based on liver plus adipose tissue measurements.

<sup>b</sup> Assuming an absolute availability from corn oil of 80%.

<sup>c</sup> Using the 0.5X dose group (Group 4) as the reference group for calculating RBA

<sup>d</sup> Using the 0.8X dose group (Group 5) as the reference group for calculating RBA

<sup>e</sup> Outlier omitted from rat RBA estimate from the pilot study; see results section of pilot study report for discussion.

## **Appendix A**

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### **Study Design Modifications for the Follow-Up to the Pilot Study of Oral Bioavailability of Dioxins/Furans in Midland Soil**



**Study Design Modifications for the  
Follow-Up to the Pilot Study of  
Oral Bioavailability of  
Dioxins/Furans in Midland Soil**

Prepared for

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## Introduction

This document describes a proposed study design for a follow-up to the pilot study of the oral bioavailability of dioxins and furans from Midland and Tittabawassee River flood-plain soils. The pilot study results showed statistically significant differences in hepatic EROD activity (a marker for cytochrome P450 1A1 induction) between the rats dosed with soils and their respective reference groups, with higher enzyme activity observed in the reference-group rats compared to the rats in the respective soil groups. This follow-up study is designed to repeat the pilot rat study, with study design modifications structured to allow an assessment of the possible impact of the differential enzyme induction on the estimation of relative bioavailability of these compounds from soil.

The observed differences in EROD activity were likely due to a difference in absorbed dose of dioxin and furan (PCDD/F) compounds (Figure 1). Rats in the corn oil reference groups received greater administered doses of PCDD/Fs than the soil/feed mixture groups, due to lower-than-expected consumption of feed by all rat groups (Table 1). In addition, the fraction of administered dose absorbed in the soil groups may have been  $\frac{1}{4}$  to  $\frac{1}{2}$  of the fraction absorbed from the corn oil gavage administration. The initial study utilized comparable corn oil and soil/feed mixture dosages of dioxins and furans, which did not take into account these two variables. The difference in EROD activity between the soil and reference groups is likely due to higher liver concentrations achieved due to higher absorbed doses of PCDD/Fs in the reference groups compared to the soil groups and resulting hepatic EROD activity.

CYP1A1 is involved in the metabolism of several of the key TCDD toxic equivalency (TEQ)-contributing compounds in the Midland and Tittabawassee River flood-plain soils, and induction of this enzyme can result in an increased rate of metabolism for these compounds. Because the method used to estimate relative bioavailability in this study relies on an assumption that the elimination rate (including elimination through metabolism and other clearance mechanisms) for each compound is the same in the soil and reference oil dose groups, demonstrated statistically significant differences in EROD activity among the groups may result in invalid estimates of relative bioavailability for any congener for which metabolism is mediated by CYP1A1. In the pilot study, estimates of relative bioavailability for many of the compounds in the study were statistically significantly different between the rats and the swine. The rats displayed different EROD activities in the soil and reference groups (while the swine did not); therefore, this factor may account for some of the observed differences in apparent relative bioavailability between the two species. Other factors related to differing tissue concentrations, including differential rates of passive elimination at different liver or body concentrations, could also lead to confounding of the interpretation of the initial pilot study results.

## Methods

This follow-up to the pilot study is designed to repeat the rat study of the Tittabawassee River flood-plain soil assessed in the pilot study. The pilot study design will be used, with key modifications designed to provide data to address the issues raised by differential EROD or MROD induction.

1. *Use of additional reference corn oil groups.* In the pilot study, the reference corn oil materials were prepared with concentrations of the key contaminants designed to result in a match to the *administered* dose of these compounds in the soil/feed mixture. In this follow-up study, the reference oil will be formulated at three doses in an attempt to bracket the anticipated *absorbed* dose of compounds from the soil/feed mixture. The purpose of this modification is to try to achieve reference corn oil dosed groups with hepatic TEQ concentrations that bracket and/or approximate the hepatic TEQ concentrations resulting from the consumption of the soil/feed mixture. This, in turn, should result in one or more reference corn oil groups with hepatic EROD and MROD activity similar to that in the soil/feed mixture group.
2. *Selection of reference corn oil dose levels.* No differential enzyme induction between experimental dose groups (reference corn oil groups vs. soil/feed groups) was observed in the swine study from either tested soil. The relative bioavailability estimates from the swine portion of the pilot study for the five tested furan compounds in the Tittabawassee River flood-plain soil ranged from a low of 0.22 for 2,3,7,8-TCDF to a high of 0.37 for 1,2,3,6,7,8-HxCDF, with a TEQ-weighted mean of 0.27. The relative bioavailability estimates in swine for the five key compounds in the Midland soil ranged from 0.18 for TCDD to 0.55 for 1,2,3,4,6,7,8-HpCDD, with a TEQ-weighted mean of 0.23 to 0.29, depending on the assumptions used for non-detectable compounds. These estimates provide a hypothesis for the level of relative bioavailability that may be observed in the absence of possible confounding from differential EROD activity. Based on this, the reference corn oil materials will be formulated to bracket the anticipated absorbed doses from the soil/feed mixture. Thus, reference corn oil mixtures will be formulated to achieve administered daily doses equal to 0.2, 0.5, and 0.8 times the administered doses in the soil/feed mixture. Because the same soils are being used as were used in the pilot study, the original reference corn oil mixture will serve as a fourth dosing level for assessment of dose-related changes in hepatic TEQ and EROD/MROD activity.
3. *Addition of undosed controls for hepatic EROD/MROD activity determination.* The relatively low levels of EROD activity observed in the pilot study raised questions on the part of the peer-review committee regarding the variability in control EROD activity. Non-simultaneous background-exposed animals from a previous phase of the project showed low levels of EROD activity, but no undosed controls were included in the pilot-study protocol. In this follow-up study, two undosed control groups (both groups fed clean feed, and one group administered corn oil gavage with no spiked dioxin or furan congeners) will be maintained for the 30-day study duration, and liver tissue will be collected at the end of the study. EROD and MROD activities will be measured in these control animals, to confirm the low activities observed in the earlier background study. These data will assist in interpreting the EROD/MROD activity data obtained from



dosed animals. Liver and adipose tissue concentrations in the each of the control rat groups (clean feed only and clean feed plus corn oil) will be measured in composited samples of livers and adipose tissues collected from five animals in each of these groups, to confirm the background tissue concentrations for use in EROD/MROD dose-response analysis.

Additional modifications unrelated to the differential EROD activity will be made based on the results of the pilot study, to streamline the study and respond to animal care issues raised in the first study:

1. In the pilot study, tissues were collected and homogenized from pairs of rats in order to collect large enough fat samples to achieve sufficiently low detection limits, to ensure detection of the administered compounds. The results of the pilot study demonstrated that the tissue concentrations (particularly in liver) in these animals easily exceeded detection limits for all congeners of relevance for both soils. For that reason, the follow-up study will analyze tissues (liver and fat) from five single animals per dose group, rather than five pairs of animals
2. Based on gavage-related mortality observed in the pilot study, seven (rather than five) rats will be included in each of the corn oil gavage groups during the compound administration phase of the study, to ensure that at least five animals reach the conclusion of the 30-day dosing period. At the end of the administration period, five rats will be selected at random from all surviving rats in each gavage group for tissue collection. Remaining rat carcasses will be frozen and stored, in case additional follow-up analyses are deemed necessary.

Tables 2 and 3 present a summary of the dose groups, dosing material analysis, and tissue analysis for the follow-up study.

As in the pilot study, the soil/feed mixture will be prepared at WIL Research. All analytical work, and the preparation of the reference corn oil dosing materials, will be conducted at Alta Analytical. Analysis of hepatic tissue samples for EROD and MROD activity will be conducted by Entrix. Animal husbandry and dosing will be conducted at the College of Veterinary Medicine at the University of Missouri—Columbia, under the direction of Dr. Stan Casteel. Other aspects of animal husbandry, diet, etc., will be conducted as described in the pilot-study report.

## Data Analysis

1. *Assessment of dose-dependence of elimination rate by congener.* Liver and adipose tissue concentration data from each animal in each of the three corn oil reference groups will be analyzed to estimate the fraction of total administered dose retained in the tissues at the end of the 30-day dosing period for each of the five target congeners. Data generated from the corn oil reference group from the original pilot study will also be included in this analysis. If there is no dose dependence of elimination rate for a given congener, the fraction of administered dose retained should be similar among all groups

regardless of administered dose. If the fraction of administered dose retained decreases with increasing administered dose, this provides evidence that the elimination rate of this congener is dose dependent in the range of doses examined.

2. *Evaluation of EROD/MROD activity as a function of hepatic TEQ.* EROD and MROD activities for all individual animals and dose groups will be plotted versus hepatic TEQ concentration. The liver-tissue concentration-response curves for EROD and MROD activity will be characterized (similar to Figure 1 of this document). The reference corn oil group(s) that provide the closest match to the EROD activity of the soil/feed group will be identified.
3. *Comparison of fraction of soil dose retained to initial pilot study.* Tissue retention and concentrations in the soil/feed mixture group will be compared to the results from the initial pilot-study Tittabawassee River flood-plain soil/feed mixture group to evaluate the degree to which the results are reproducible from experiment to experiment.
4. *RBA calculation.* The relative bioavailability of the contaminants from the soil/feed mixture will be estimated using the same calculation procedures outlined in the pilot-study report. However, these calculations will be presented based only on the one or two reference corn oil group(s) with hepatic TEQ and EROD activities that are most similar to those of the soil/feed mixture group, as identified in step 2 above.

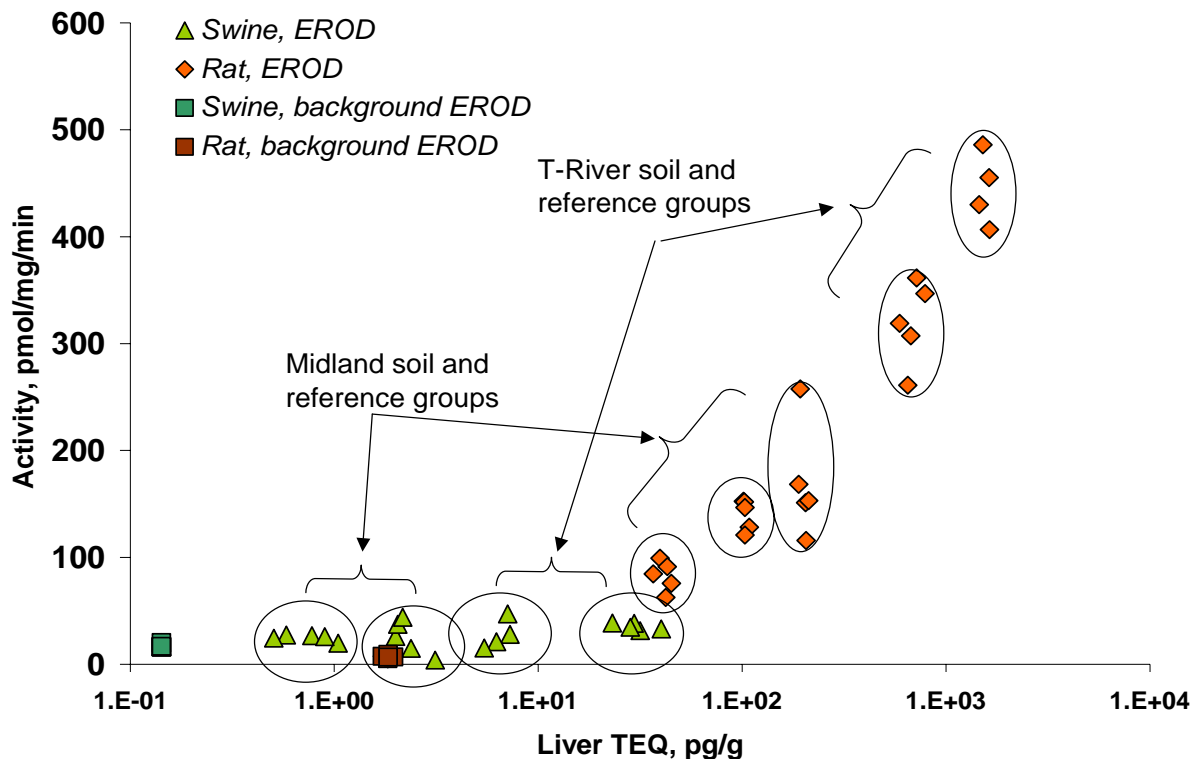


Figure 1. EROD activity as a function of liver TEQ concentration for the rat and swine experimental groups in the oral bioavailability pilot study. While the swine demonstrated no statistically significant differences in hepatic EROD activity between reference oil and soil groups, such statistically significant differences were observed in the rat groups, with reference oil and feed groups demonstrating elevated liver TEQ and EROD activity compared to soil groups for both soils. There was no overlap in the EROD activity or hepatic TEQ concentrations between soil and reference oil groups for either soil.

**Table 1. Comparison of administered doses and hepatic TEQ concentrations in rat study groups in initial pilot study**

Dose Group	Admin. Dose (ng TEQ/kg-d)	Hepatic TEQ (pg/g)	#-Fold Difference Compared to Soil Group	
			Admin. Dose	Hepatic TEQ
Midland Soil/Feed	0.6	41	--	--
Ref. Feed	0.7	104	1.2	2.5
Ref. Oil Gavage	1.0	201	1.7	4.9
T-River Soil/Feed	2.6	684	--	--
Ref. Oil Gavage	2.9	1556	1.1	2.3

**Table 2. Summary of dose groups for follow-up study**

Group	Description	Number of Animals in Test	HR-GC/MS Analysis		
			Liver	Adipose	EROD/MROD Analysis
FC	Feed control	5	1 <sup>a</sup>	1 <sup>a</sup>	5
GC	Corn oil gavage control	7	1 <sup>a</sup>	1 <sup>a</sup>	5
SF	Tittabawassee River soil/feed mixture, nominal daily dose rate Y	5	5	5	5
G1	Reference corn oil spiked at 0.2×Y	7	5 <sup>b</sup>	5 <sup>b</sup>	5
G2	Reference corn oil spiked at 0.5×Y	7	5 <sup>b</sup>	5 <sup>b</sup>	5
G3	Reference corn oil spiked at 0.8×Y	7	5 <sup>b</sup>	5 <sup>b</sup>	5
<b>Totals:</b>		38	22	22	30

<sup>a</sup> Liver tissue samples from five animals in each of the control groups will be collected and composited for HR-GC/MS analysis, to confirm liver tissue concentrations at background levels for use in EROD/MROD dose-response analysis.

<sup>b</sup> Five animals randomly selected from all remaining group animals at the end of the 30-day dosing period.

**Table 3. Summary of samples for HR-GC/MS analysis**

<b>Sample Description</b>	<b>Number of Analyses</b>
Soil/feed mixture, pre-test characterization, triplicate split sample for analysis	3 <sup>a</sup>
Soil/feed mixture, post-administration for confirmation of stability	1
Unspiked corn oil, pre-test confirmation of lack of dioxin/furan contamination	1
Reference corn oil solutions, pre-test characterization for confirmation of compound concentrations	3 <sup>a</sup>
Reference corn oil solutions, post-administration for confirmation of stability	3
Liver tissue samples, five each from four dose groups plus 1 composited liver tissue sample from each of the two control groups	22
Adipose tissue samples, five each from four dose groups	22

<sup>a</sup> These analyses will be requested on a “rush” basis, in order to prepare dosing solutions and feed mixtures in a compressed time frame.

## **Appendix B**

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**WIL Research Report:  
Preparation of Diets for a  
Dietary Exposure Study with  
Dioxin-Contaminated Soils in  
Rats**

**PROJECT TITLE**

Preparation of Diets for a Dietary Exposure Study with a Dioxin-Contaminated  
Soil in Rats

**PROJECT NUMBER**

WIL-518002

**CONTRIBUTING SCIENTIST**

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### GENERAL CONSIDERATIONS

To my knowledge, there were no significant deviations from the intended scope of work or the Standard Operating Procedures of WIL Research Laboratories, LLC that would be expected to affect the scientific integrity of this study.

  
Daniel W. Sved, Ph.D.  
Director, Metabolism and  
Analytical Chemistry

12 January 2006  
Date



## **PREPARATION OF DIETS FOR A DIETARY EXPOSURE STUDY WITH A DIOXIN-CONTAMINATED SOIL IN RATS**

### **1. INTRODUCTION**

WIL Research Laboratories, LLC was subcontracted by Exponent, Inc. to prepare a rodent diet containing 5% of a test soil and to provide additional basal rodent diet. Samples of the dietary admixture were sent to Alta Analytical Laboratory for analysis. The dietary admixture and basal diet were shipped to the Veterinary Medical Diagnostic Laboratory, University of Missouri-Columbia.

### **2. TEST MATERIALS**

The following material was supplied to WIL Research Laboratories for use in preparing the dietary admixture.

#### **A. Test Soil**

The test soil was received from Exponent, Inc., Boulder, CO on November 9, 2005 and was assigned WIL Log No. 6705A. The material was labeled with the following information.

061804-SOI-02769-00.5  
Lot# DPW  
Sampling Site: THT02769 (IP-2)  
Sample Type Other <250  $\mu$ m  
455 g  
Bottle Archive 3 of 3  
Tag No. 59512

### **3. BASAL DIET**

The basal diet used for this project was PMI International, LLC Certified Rodent LabDiet 5001 (meal). Lot number OCT 26 05 1 was used for the dietary admixture, which was prepared on November 16, 2005; the remaining diet from this lot was shipped to the Veterinary Medical Diagnostic Laboratory, University of Missouri-Columbia.

### **4. MIXING PROCEDURE**

A total batch size of 5 kg was prepared. The required amount of test soil, 250 g, was weighed into a tared vessel. A pre-mixture was prepared by transferring the test soil to a Hobart mixer containing 1000 g of basal diet and the

components were mixed for 5 minutes with the speed setting on 1. The pre-mixture was transferred to a V-blender along with the remaining amount of basal diet (3750 g) needed to achieve the total batch size. The components were mixed for 15 minutes using the intensifier bar for the first and last 5 minutes.

## **5. SAMPLE COLLECTION AND SHIPMENT**

Three samples (approximately 100 g each) of the dietary admixture were collected into plastic ziplock-type bags. Samples were collected from the initial (bottom), middle, and last (top) portions of the admixture as it was discharged from the V-blender. Samples were shipped under ambient conditions to Alta Analytical Laboratory using an overnight courier on November 16, 2005.

## **6. SHIPMENT OF DIETARY ADMIXES**

Upon receiving authorization from Exponent, the dietary admixture and remaining basal diets were shipped under ambient conditions to the Veterinary Medical Diagnostic Laboratory, University of Missouri-Columbia using an overnight courier. Additionally, the remaining basal diet (three boxes each containing 22.67 kg of lot number OCT 26 05 1) was also shipped.

## **7. DISPOSITION OF REMAINING TEST MATERIALS**

Following shipment of the dietary admixture, any remaining test soil was returned to the supplier.

## **Appendix C**

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### **Entrix Report**

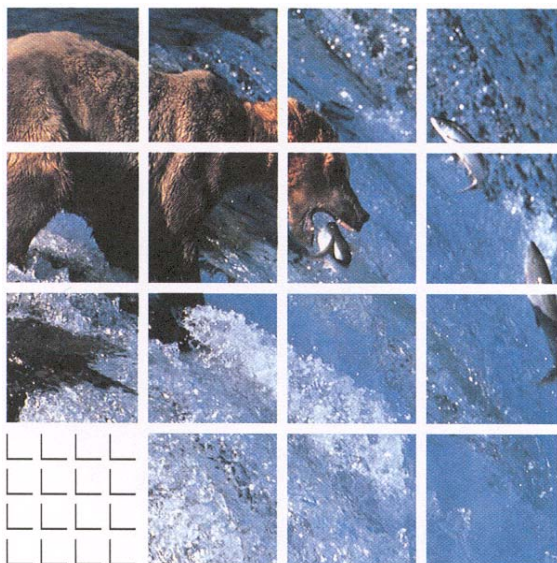
**QUANTIFICATION OF EROD AND MROD ACTIVITIES IN RAT LIVER  
MICROSOMES: FEBRUARY 22, 2006 SAMPLES**

*Prepared for:*

**Exponent  
Colleen Cushing**

*Prepared by:*

**ENTRIX, Inc.**  
John L. Newsted, Ph.D.  
John P. Giesy, Ph.D.



March, 2006

## Overview

This interim report summarizes the results of the analysis of Ethoxyresorufin *O*-deethylase (EROD) and Methoxyresorufin *O*-demethylase activity in the liver microsomes of rats. Liver samples were collected from rats feed as part of a study to evaluate the bioavailability of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) from soils to organisms consuming soil. The protocols used to prepare liver microsomes and to measure both the protein levels and the enzymatic activities are outlined in the MSU-ATL SOP# 250, version 1.1 (Protocol for Liver Microsome Preparation and Microsomal Protein Measurement and AROD Assays in the same 96-Well Plate).

## Methods

Rat livers were collected on 2-22-2006, frozen in liquid nitrogen and shipped to Entrix for processing. Samples were received by Entrix on 2-23-2006 and immediately sent to Michigan State University-Aquatic Toxicology Laboratory and stored in liquid nitrogen until processed. The dates for the preparation of liver microsomes preparation are shown in Table 1.

**Table 1.** Rat liver samples and dates of Microsomal preparation for use in analysis of cytochrome P450 activities.<sup>a</sup>

Preparation Date	Rat Liver Samples
3/6/2006	14, 15, 17, 18, 20, 22
3/7/2006	23, 24, 26, 27, 29, 30, 32, 35, 36, 37, 38, 39
3/8/2006	40, 41, 42, 43, 44, 46, 47, 48, 50, 51, 53, 54

<sup>a</sup> Microsomes were processed and then stored at -80°C until EROD and MROD analysis

As outlined in SOP# 250, sets of proteins and resorufin standards were run with each microtiter plate to account for differences in assay conditions and instrumental performance. All Microsomal samples were thawed and stored on ice (4°C) prior to the start of the enzyme assays. All working solutions including resorufin standards, 7-ethoxyresorufin (7-ER) and 7-methoxyresorufin (7-MR) and NADPH solutions were prepared the day of the assay and stored on ice prior to use. Incubation conditions and enzymatic substrate concentrations for the rat EROD and MROD assays are given below:

Pre-incubation time: 10 min @ 37°C  
Incubation time: 10 min @ 37°C

Final Substrate Concentrations:

7-ER 2.5 µM  
7-MR 5.0 µM

Fluorescence Filter Settings:

AROD: Excitation - 538 nm  
Emission - 590 nm  
Protein: Excitation - 355 nm  
Emission - 460 nm

EROD/MROD activities and protein concentrations were measured within the same wells in a 96-well plate. Protein concentrations were measured by a fluorometric method at the end of incubation time and differences between animals and replicates were taken into account during the analysis of the data. Fluorescence was measured with a Fluoroskan Ascent 2.5 multiplate reader (Thermo Electron Corp.) and the data was electronically collected and stored as an Excel file (\*.xls). Protein concentrations and enzymatic activities were calculated using Excel (Office 2003). In addition, all descriptive statistics were calculated in Excel. These files have been attached to this report in Appendices A (EROD) and B (MROD).

## **Results**

All rat liver samples were analyzed for EROD and MROD on 3-21-06 while proteins were determined on 3-22-06 (Table 2). For the EROD analyses, the intra-sample variability across all groups was relatively low and coefficients of variation (CVs) ranged from 0.63% to 7.52% with an average value of 3.06%. The intra-group variability for EROD was slightly greater than that observed for within samples and the CVs ranged from 15% to 22% with an average value of 18%. The variability observed in the MROD analyses was slightly greater than that observed in the EROD results. For MROD, the intra-sample CVs ranged from 0.38% to 8.2% with an average value of 5.0% across all samples. The intra-group variability was much greater than the intra-sample variability in that group CVs ranged from 17% to 46% and averaged 28% for all groups.

There was an increase in EROD activity when evaluated by groups with the least activity being observed in Group 1 while the greatest was observed in Group 5 where average EROD activities were 33.2 and 106 pmol/mg protein/min, respectively. The activity in Group 6 appeared to have reached a plateau and did not differ from that observed in Group 5 samples.

The general trend in MROD activity was similar to that observed for EROD where the least activities were measured in Groups 1 and 2 followed by an increase in activity up to a maximal level in samples from Group 5. There was approximately a 39% decrease in the measured MROD activity between Groups 5 and 6.

## **Conclusions**

Assays were conducted with microsomes prepared from rat livers to measure the activity of two cytochrome P450s, P450 1A1 (EROD) and P450 1A2 (MROD). The overall variability in EROD and MROD activity measured either on a sample basis or on a group was similar with intra-sample variability was on average, less than 5%. Intra-group as determined by differences in measured values within a group was greater than that observed within a sample and averaged approximately 18% and 28% for EROD and MROD, respectively. Activity of both enzymes increased across the groups with the least enzymatic activity being observed in Group 1 rats and the greatest activity being observed in Group 5 rats. For Group 6 rats, EROD activity did not increase but was equivalent to that measured in Group 5 rats while for MROD, the activity in Group 6 rats was approximately 39% less than that measured in Group 5 rats.

**Table 2.** Mixed function oxygenase activities in rat liver samples. <sup>a</sup>

Group	Sample	EROD (pmol/mg/min)	MROD (pmol/mg/min)
Gp-1	17	31.5 ± 0.43	26.9 ± 2.26
	18	25.4 ± 0.82	27.7 ± 1.03
	20	32.3 ± 0.38	26.6 ± 1.08
	44	42.4 ± 2.29	22.0 ± 1.05
	46	34.1 ± 0.40	25.3 ± 1.05
	<b>Group Average</b>	<b>33.2 ± 6.13</b>	<b>25.7 ± 2.2</b>
Gp-2	32	33.5 ± 1.04	29.3 ± 1.18
	35	33.4 ± 0.88	26.5 ± 2.16
	38	44.2 ± 0.45	24.4 ± 0.30
	40	49.9 ± 0.85	26.9 ± 1.63
	54	42.2 ± 0.88	27.5 ± 1.43
	<b>Group Average</b>	<b>40.6 ± 7.15</b>	<b>26.9 ± 1.8</b>
Gp-3	22	42.3 ± 1.25	36.3 ± 1.56
	24	49.3 ± 1.38	36.1 ± 2.27
	37	54.3 ± 0.52	34.8 ± 2.47
	47	61.2 ± 1.99	31.5 ± 1.87
	50	62.1 ± 0.99	28.0 ± 0.97
	<b>Group Average</b>	<b>53.6 ± 8.07</b>	<b>33.3 ± 3.6</b>
Gp-4	14	73.3 ± 2.52	31.3 ± 1.30
	23	83.6 ± 4.53	51.2 ± 0.30
	39	110 ± 8.26	36.5 ± 0.58
	43	74.7 ± 2.03	24.8 ± 1.36
	53	62.6 ± 1.91	30.9 ± 1.76
	<b>Group Average</b>	<b>80.8 ± 17.9</b>	<b>34.9 ± 10</b>
Gp-5	15	115 ± 4.84	52.0 ± 2.78
	26	120 ± 4.04	36.6 ± 2.40
	27	117 ± 6.76	39.3 ± 1.32
	29	100 ± 3.54	47.0 ± 3.59
	36	80.0 ± 3.55	34.5 ± 2.62
	<b>Group Average</b>	<b>106 ± 16.6</b>	<b>41.9 ± 7.4</b>
Gp-6	30	82.0 ± 1.89	33.6 ± 2.67
	41	118 ± 4.67	39.0 ± 1.02
	42	143 ± 8.34	30.1 ± 1.43
	48	116 ± 0.73	41.2 ± 1.93
	51	91.1 ± 1.00	28.7 ± 1.37
	<b>Group Average</b>	<b>110 ± 24.1</b>	<b>34.5 ± 5.5</b>

<sup>a</sup> Activities given as means and standard deviations. Each sample was analyzed in triplicate.

## **APPENDICES**



## **Appendix A: Chain of Custody and Data Sheets**

**Jan-06**

[illegible]

## **Appendix B: EROD Excel Spreadsheets**

## Original Data

EROD #1															
Measurement count: 1	Ex: 538	Em: 590	Scaling Factor : 1/1												
Temp(°C)	25.7	1	1	2	3	4	5	6	7	8	9	10	11	12	
							2.541	65.32	65.84	64.86	2.618	82.13	82.27	86	
				1.108	14.93	109.6	2.119	82.56	82.27	85.69	3.134	72.3	76.66	78.68	
				1.096	15.05	109.3	1.625	30.67	31.03	29.2	2.27	73.44	69.91	78.39	
				1.082	14.88	108.7	1.652	20.22	21.18	20.11	2.307	59.11	59.35	61.22	
				8.21	54.8	147.5	1.638	27.69	25.64	26.33	1.694	24.43	25.62	23.14	
				8.234	54.77	148.4	1.916	36.69	38.05	36.03	1.654	22.06	21.95	22.83	
				8.258	54.66	149.7	2.172	45.69	45.08	44.67	2.487	48.3	49.68	52.68	
							1.859	35.76	34.53	34.21	1.981	38.96	38.83	40.85	
Protein #1															
Measurement count: 1	Ex: 355	Em: 460	Scaling Factor : 1/1												
			1	2	3	4	5	6	7	8	9	10	11	12	
							587.5	678.1	656.1	640.3	525.7	554.3	544.6	546.4	
				140	199.9	332.7	501.9	575.5	563.4	557.2	554.5	578.9	581.1	587.6	
				141.1	206.3	334	672.4	692.3	697.2	651.5	456.1	524.9	497.3	509.2	
				140.7	208.4	335.4	539.3	584.8	599.5	555.9	525.8	569.3	556.7	565.9	
				171.3	274.9	369.3	555.4	617.7	586.1	588.3	431.9	546.3	552	532.6	
				172.7	273.6	371.1	638.6	647	636.7	623.8	451.6	512.4	503.1	507	
				170.3	268.9	368.7	530.1	556	470.1	434.1	437.1	504.5	499.2	505	
EROD #2															
Measurement count: 1	Ex: 538	Em: 590	Scaling Factor : 1/1												
Temp(°C)	25.7	1	1	2	3	4	5	6	7	8	9	10	11	12	
							1.496	17.54	17.59	17.16	1.771	34.41	33.24	35.05	
				1.064	15.07	108	1.876	49	49.15	45.03	2.24	66.59	64.75	66	
				1.054	15.05	109.6	1.346	27.66	28.85	29.47	1.569	36.63	37.99	38.67	
				1.059	14.91	109	2.222	65.65	67.04	69.01	2.242	54.02	57.36	59.37	
				8.278	54.22	147.9	2.255	79.12	79.15	75.93	1.792	34.7	36.25	39.01	
				8.255	55.4	150.2	1.967	50.61	51.47	55.09	1.505	25.05	26.04	26.14	
				8.342	54.45	148.8	1.475	22.32	21.79	21.74	1.582	1.132	1.217	1.127	
							1.445	24.91	24.51	25.11	1.488	1.162	1.156	1.087	
Protein #2															
Measurement count: 1	Ex: 355	Em: 460	Scaling Factor : 1/1												
			1	2	3	4	5	6	7	8	9	10	11	12	
							363.7	396.3	395.4	393.5	491.8	509.1	519.3	525	
				127.3	249.2	336.9	424	436.2	435.8	448.8	523.4	533.6	526.6	534.4	
				128.5	256.1	341.1	499.5	512	530.3	527.1	543.5	545.6	553.4	574.4	
				123.5	253.4	335.7	516.3	511.2	530.9	561.4	548.8	551.9	573	583.5	
				181.5	306.2	553.9	501.1	504.6	525.4	532.1	517.1	522.1	537.3	548.3	
				180.4	307.2	552.9	621.9	608.5	620.1	629.8	527	536.6	553.7	540.7	
				179.3	301.6	556	552.3	563.2	553.4	560.2	4.203	127.4	134	135.2	
							538.2	539.6	533.1	508.5	7.78	137	135.2	130.6	

## Data &amp; IDs

## EROD PLATE 1

## Set 1: EROD Fluorescence Readings

	1	2	3	4	5	6	7	8	9	10	11	12
A					2.541	65.32	65.84	64.86	2.618	82.13	82.27	86.0
B		1.108	14.93	109.6	2.119	82.56	82.27	85.69	3.134	72.3	76.66	78.68
C		1.096	15.05	109.3	1.625	30.67	31.03	29.2	2.27	73.44	69.91	78.39
D		1.082	14.88	108.7	1.652	20.22	21.18	20.11	2.307	59.11	59.35	61.22
E		8.21	54.8	147.5	1.638	27.69	25.64	26.33	1.694	24.43	25.62	23.14
F		8.234	54.77	148.4	1.916	36.69	38.05	36.03	1.654	22.06	21.95	22.83
G		8.258	54.66	149.7	2.172	45.69	45.08	44.67	2.487	48.3	49.68	52.68
H					1.859	35.76	34.53	34.21	1.981	38.96	38.83	40.85

## Set 2: Protein Fluorescence Readings

	1	2	3	4	5	6	7	8	9	10	11	12
A					587.5	678.1	656.1	640.3	525.7	554.3	544.6	546.4
B		140	199.9	332.7	501.9	575.5	563.4	557.2	554.5	578.9	581.1	587.6
C		141.1	206.3	334	672.4	692.3	697.2	651.5	456.1	524.9	497.3	509.2
D		140.7	208.4	335.4	539.3	584.8	599.5	555.9	525.8	569.3	556.7	565.9
E		171.3	274.9	369.3	555.4	617.7	586.1	588.3	431.9	546.3	552	532.6
F		172.7	273.6	371.1	638.6	647	636.7	623.8	451.6	512.4	503.1	507
G		170.3	268.9	368.7	393.4	452.9	470.1	434.1	437.1	504.5	499.2	505
H					530.1	556	546.2	521.7	471.4	553.2	549.1	566

## Data & IDs

Entrix		Exponent	
Cells	Sample ID	Group	Sample
A5-8	S14	Gp-4	14
B5-8	S15	Gp-5	15
C5-8	S17	Gp-1	17
D5-8	S18	Gp-1	18
E5-8	S20	Gp-1	20
F5-8	S22	Gp-3	22
G5-8	S23	Gp-4	23
H5-8	S24	Gp-3	24
A9-12	S26	Gp-5	26
B9-12	S29	Gp-5	29
C9-12	S27	Gp-5	27
D9-12	S30	Gp-6	30
E9-12	S32	Gp-2	32
F9-12	S35	Gp-2	35
G9-12	S36	Gp-5	36
H9-12	S37	Gp-3	37

## Data &amp; IDs

## EROD PLATE 2

## Set 1: EROD Fluorescence Readings

	1	2	3	4	5	6	7	8	9	10	11	12
A					1.496	17.54	17.59	17.16	1.771	34.41	33.24	35.05
B		1.064	15.07	108	1.876	49	49.15	45.03	2.24	66.59	64.75	66
C		1.054	15.05	109.6	1.346	27.66	28.85	29.47	1.569	36.63	37.99	38.67
D		1.059	14.91	109	2.222	65.65	67.04	69.01	2.242	54.02	57.36	59.37
E		8.278	54.22	147.9	2.255	79.12	79.15	75.93	1.792	34.7	36.25	39.01
F		8.255	55.4	150.2	1.967	50.61	51.47	55.09	1.505	25.05	26.04	26.14
G		8.342	54.45	148.8	1.475	22.32	21.79	21.74				
H					1.445	24.91	24.51	25.11				

## Set 2: Protein Fluorescence Readings

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	0	363.7	396.3	395.4	393.5	491.8	509.1	519.3	525.0
B	0	127.3	249.2	336.9	424	436.2	435.8	448.8	523.4	533.6	526.6	534.4
C	0	128.5	256.1	341.1	499.5	512	530.3	527.1	543.5	545.6	553.4	574.4
D	0	123.5	253.4	335.7	516.3	511.2	530.9	561.4	548.8	551.9	573	583.5
E	0	181.5	306.2	553.9	501.1	504.6	525.4	532.1	517.1	522.1	537.3	548.3
F	0	180.4	307.2	552.9	621.9	608.5	620.1	629.8	527	536.6	553.7	540.7
G	0	179.3	301.6	556	552.3	563.2	553.4	560.2				
H	0	0	0	0	538.2	539.6	533.1	508.5				

## Data & IDs

Entrix		Exponent	
Cells	Sample ID	Group	Sample
A5-8	S38	Gp-2	38
B5-8	S39	Gp-4	39
C5-8	S40	Gp-2	40
D5-8	S41	Gp-6	41
E5-8	S42	Gp-6	42
F5-8	S43	Gp-4	43
G5-8	S44	Gp-1	44
H5-8	S46	Gp-1	46
A9-12	S47	Gp-3	47
B9-12	S48	Gp-6	48
C9-12	S50	Gp-3	50
D9-12	S51	Gp-6	51
E9-12	S53	Gp-4	53
F9-12	S54	Gp-2	54
G9-12			
H9-12			

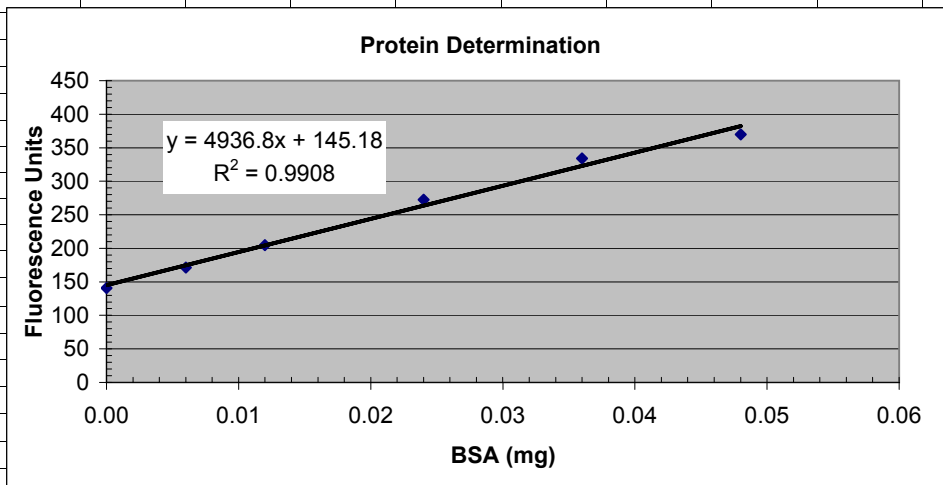


## EROD#1 Analysis

[illegible]

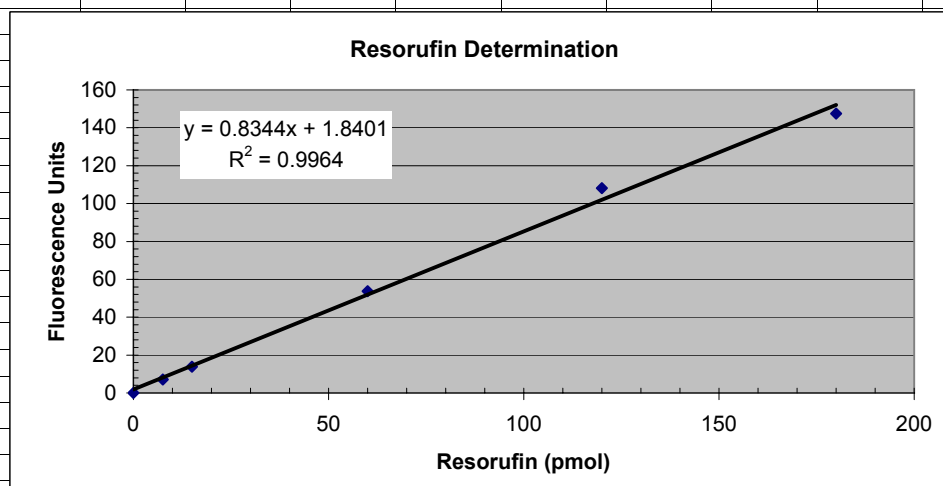
**Protein Determination**

BSA (mg)	Mean Fluor.
0.00	141
0.006	171
0.012	205
0.024	272
0.036	334
0.048	370



**Resorufin Determination**

Resorufin (pmol)	Mean Fluor.	Adj. Fluor.
0	1.10	0.007
7.5	8.2	7.1
15	15.0	13.9
60	54.7	53.6
120	109.2	108.1
180	148.5	147.4



## EROD#1 Analysis

Set 1: Resorufin Content (pmol)												
	1	2	3	4	5	6	7	8	9	10	11	12
A					0.8	76.1	76.7	75.5	0.9	96.2	96.4	100.9
B					0.3	96.7	96.4	100.5	1.6	84.4	89.7	92.1
C					-0.3	34.6	35.0	32.8	0.5	85.8	81.6	91.7
D					-0.2	22.0	23.2	21.9	0.6	68.6	68.9	71.2
E					-0.2	31.0	28.5	29.4	-0.2	27.1	28.5	25.5
F					0.1	41.8	43.4	41.0	-0.2	24.2	24.1	25.2
G					0.4	52.6	51.8	51.3	0.8	55.7	57.3	60.9
H					0.0	40.7	39.2	38.8	0.2	44.5	44.3	46.8
y = 1.7073x + 30.29		X=	0.8344									
		Intercept=	1.8401									
Set 2: Protein Concentration (mg)												
	1	2	3	4	5	6	7	8	9	10	11	12
A					0.090	0.108	0.103	0.100	0.077	0.083	0.081	0.081
B					0.072	0.087	0.085	0.083	0.083	0.088	0.088	0.090
C					0.107	0.111	0.112	0.103	0.063	0.077	0.071	0.074
D					0.080	0.089	0.092	0.083	0.077	0.086	0.083	0.085
E					0.083	0.096	0.089	0.090	0.058	0.081	0.082	0.078
F					0.100	0.102	0.100	0.097	0.062	0.074	0.073	0.073
G					0.050	0.062	0.066	0.059	0.059	0.073	0.072	0.073
H					0.078	0.083	0.081	0.076	0.066	0.083	0.082	0.085
y = 20735x + 194.95		X=	4936.8									
		Intercept=	145.18									
	Not used for STDs and/or samples											

## EROD#1 Analysis

[illegible]

## EROD#2 Analysis

**Samples:** Liver Microsomes Processed on 3/6 to 3/8, 2006  
**Analysis:** EROD analyses conducted on 03-214-2006  
**Plate # 2**

### Set 1: EROD Fluorescence Readings

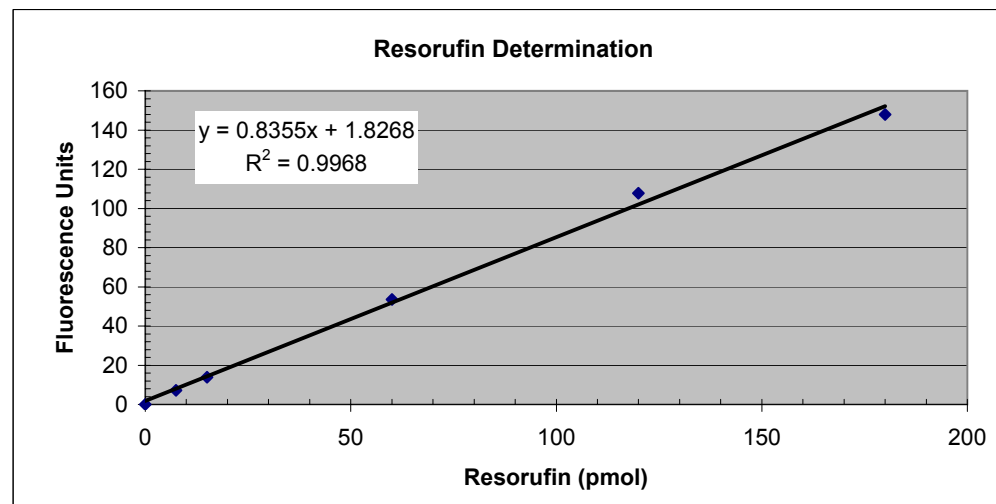
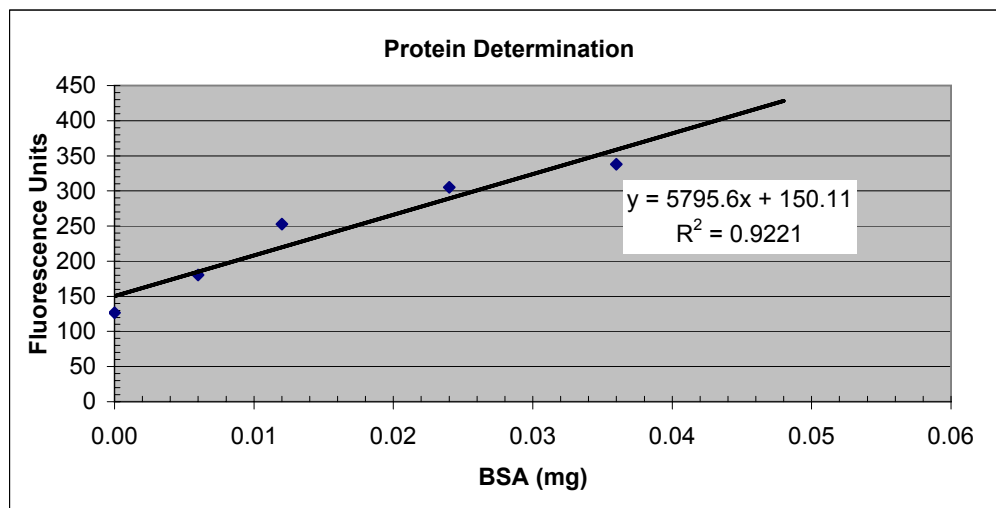
	1	2	3	4	5	6	7	8	9	10	11	12
A					1.50	17.54	17.59	17.16	1.77	34.41	33.24	35.05
B		1.06	15.07	108.00	1.88	49.00	49.15	45.03	2.24	66.59	64.75	66.00
C		1.05	15.05	109.60	1.35	27.66	28.85	29.47	1.57	36.63	37.99	38.67
D		1.06	14.91	109.00	2.22	65.65	67.04	69.01	2.24	54.02	57.36	59.37
E		8.28	54.22	147.90	2.26	79.12	79.15	75.93	1.79	34.70	36.25	39.01
F		8.26	55.40	150.20	1.97	50.61	51.47	55.09	1.51	25.05	26.04	26.14
G		8.34	54.45	148.80	1.48	22.32	21.79	21.74				
H					1.45	24.91	24.51	25.11				


**Protein Determination**

BSA (mg)	Mean Fluor.
0.00	126
0.006	180
0.012	253
0.024	305
0.036	338
0.048	554

**Resorufin Determination**

Resorufin (pmol)	Mean Fluor.	Adj. Fluor.
0	1.06	0
7.5	8.3	7.2
15	15.0	14.0
60	54.7	53.6
120	108.9	107.8
180	149.0	147.9



# EROD#2 Analysis

## Set 1: Resorufin Content (pmol)

	1	2	3	4	5	6	7	8	9	10	11	12
A					-0.4	18.8	18.9	18.4	-0.1	39.0	37.6	39.8
B					0.1	56.5	56.6	51.7	0.5	77.5	75.3	76.8
C					-0.6	30.9	32.3	33.1	-0.3	41.7	43.3	44.1
D					0.5	76.4	78.1	80.4	0.5	62.5	66.5	68.9
E					0.5	92.5	92.5	88.7	0.0	39.3	41.2	44.5
F					0.2	58.4	59.4	63.8	-0.4	27.8	29.0	29.1
G					-0.4	24.5	23.9	23.8				
H					-0.5	27.6	27.1	27.9				

$$y = 1.7073x + 30.29$$

X= 0.8355  
Intercept= 1.8268

## Set 2: Protein Concentration (mg)

	1	2	3	4	5	6	7	8	9	10	11	12
A					0.037	0.042	0.042	0.042	0.059	0.062	0.064	0.065
B					0.047	0.049	0.049	0.052	0.064	0.066	0.065	0.066
C					0.060	0.062	0.066	0.065	0.068	0.068	0.070	0.073
D					0.063	0.062	0.066	0.071	0.069	0.069	0.073	0.075
E					0.061	0.061	0.065	0.066	0.063	0.064	0.067	0.069
F					0.081	0.079	0.081	0.083	0.065	0.067	0.070	0.067
G					0.069	0.071	0.070	0.071				
H					0.067	0.067	0.066	0.062				

$$y = 20735x + 194.95$$

X= 5795.6  
Intercept= 150.11

Not used for STDs and/or samples

EROD#2 Analysis

**EROD Activity (pmmol/min/mg)**

	1	2	3	4	5	6	7	8	9	10	11	12
A						44.3	44.6	43.7		63.0	59.0	61.5
B						114.4	114.9	100.3		117.1	115.9	115.8
C						49.5	49.3	50.9		61.0	62.2	60.2
D						122.6	118.8	113.3		90.1	91.1	92.1
E						151.2	142.9	134.6		61.3	61.7	64.8
F						73.8	73.3	77.0		41.7	41.6	43.2
G						34.4	34.3	33.7				
H						41.1	41.1	45.1				

**Assay Time:** 10 min

**EROD Activity (pmmol/min/mg)**

Cells	Sample ID	Blank	Raw			Adjusted			Statistics		
			Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Mean	SD	CV (%)
A5-8	S38	0.0	44.3	44.6	43.7	44.3	44.6	43.7	44.2	0.4	1.0
B5-8	S39	0.0	114.4	114.9	100.3	114.4	114.9	100.3	109.9	8.26	7.5
C5-8	S40	0.0	49.5	49.3	50.9	49.5	49.3	50.9	49.9	0.85	1.7
D5-8	S41	0.0	122.6	118.8	113.3	122.6	118.8	113.3	118.2	4.67	4.0
E5-8	S42	0.0	151.2	142.9	134.6	151.2	142.9	134.6	142.9	8.34	5.8
F5-8	S43	0.0	73.8	73.3	77.0	73.8	73.3	77.0	74.7	2.03	2.7
G5-8	S44	0.0	34.4	34.3	33.7	34.4	34.3	33.7	34.1	0.40	1.2
H5-8	S46	0.0	41.1	41.1	45.1	41.1	41.1	45.1	42.4	2.29	5.4
A9-12	S47	0.0	63.0	59.0	61.5	63.0	59.0	61.5	61.2	1.99	3.3
B9-12	S48	0.0	117.1	115.9	115.8	117.1	115.9	115.8	116.3	0.73	0.6
C9-12	S50	0.0	61.0	62.2	60.2	61.0	62.2	60.2	61.2	0.99	1.6
D9-12	S51	0.0	90.1	91.1	92.1	90.1	91.1	92.1	91.1	1.00	1.1
E9-12	S53	0.0	61.3	61.7	64.8	61.3	61.7	64.8	62.6	1.91	3.0
F9-12	S54	0.0	41.7	41.6	43.2	41.7	41.6	43.2	42.2	0.88	2.1
G9-12											
H9-12											

Sample Identifications (IDs) can be found in Laboratory Book (Dow#1)



## Summary

### Summary of EROD Results

Entrix	Exponent		Statistics			Group Statistics	
Sample ID	Group	Sample	Mean	Stdev	CV (%)	Mean	Stdev
S17	Gp-1	17	31.5	0.43	1.37	33.2	6.13
S18	Gp-1	18	25.4	0.82	3.21		
S20	Gp-1	20	32.3	0.38	1.18		
S46	Gp-1	46	42.4	2.29	5.40		
S44	Gp-1	44	34.1	0.40	1.17		
S32	Gp-2	32	33.5	1.04	3.10	40.6	7.15
S35	Gp-2	35	33.4	0.88	2.64		
S38	Gp-2	38	44.2	0.45	1.01		
S40	Gp-2	40	49.9	0.85	1.69		
S54	Gp-2	54	42.2	0.88	2.10		
S22	Gp-3	22	42.3	1.25	2.95	53.6	8.07
S24	Gp-3	24	49.3	1.38	2.79		
S37	Gp-3	37	54.3	0.52	0.95		
S47	Gp-3	47	61.2	1.99	3.25		
S50	Gp-3	50	61.2	0.99	1.62		
S14	Gp-4	14	73.3	2.52	3.43	80.8	17.9
S23	Gp-4	23	83.6	4.53	5.42		
S39	Gp-4	39	109.9	8.26	7.52		
S43	Gp-4	43	74.7	2.03	2.71		
S53	Gp-4	53	62.6	1.91	3.05		
S15	Gp-5	15	115.1	4.84	4.21	106.4	16.6
S26	Gp-5	26	119.8	4.04	3.37		
S27	Gp-5	27	116.8	6.76	5.79		
S29	Gp-5	29	100.1	3.54	3.53		
S36	Gp-5	36	80.0	3.55	4.43		
S30	Gp-6	30	82.0	1.89	2.31	110.1	24.1
S41	Gp-6	41	118.2	4.67	3.95		
S42	Gp-6	42	142.9	8.34	5.84		
S48	Gp-6	48	116.3	0.73	0.63		
S51	Gp-6	51	91.1	1.00	1.09		

## **Appendix C: MROD Excel Spreadsheets**

## Original Data

MROD #1															
Measurement count: 1	Ex: 538	Em: 590	Scaling Factor : 1/1												
Temp(°C)	25.7	1	1	2	3	4	5	6	7	8	9	10	11	12	
							2.685	28.32	29.91	30.89	2.709	26.03	26.06	29.25	
				1.919	14.33	98.25	2.577	38.33	36.98	35.74	2.647	29.1	29.12	33.74	
				1.858	14.32	98.43	2.474	23.78	26.03	25.2	2.857	30.21	31.42	34.44	
				1.919	14.19	98.08	2.566	21.01	22.67	21.73	2.685	22.71	26.11	26.32	
				8.182	50.04	132.9	2.517	20.53	21.18	20.92	2.656	20.15	20.43	22.14	
				8.382	49.92	133.2	2.712	29.23	32.11	26.92	2.637	16.55	17.38	19.07	
				8.466	49.94	133.8	2.635	27.42	27.15	26.35	2.736	21.35	21.83	24.51	
							2.666	27.24	22.91	26.04	2.55	24.17	26.68	29.27	
Protein #1															
Measurement count: 1	Ex: 355	Em: 460	Scaling Factor : 1/1												
			1	2	3	4	5	6	7	8	9	10	11	12	
							603.1	580.2	603.1	586.9	448.5	492.8	483.3	493.1	
				117.7	244.1	333.3	460.4	474.8	488.8	483.8	421.2	436.8	457.1	458.4	
				119.5	248.6	333.3	570.6	595.1	584.8	551	493.8	517.1	524	545.2	
				117.6	246	334.1	482.9	505.4	508.2	504.7	422.1	486.5	495	488.9	
				177.9	302.7	550.3	509.7	509.3	513.4	488.8	432.6	460	475.2	477.7	
				179.7	299.8	547.6	476.3	525.3	542.8	503.8	318.9	442.6	446.9	443.3	
				179.3	301	559.3	387.2	395.1	392.5	382.2	407.8	436.9	451.1	446.8	
							450.4	490.9	459.2	463.7	476.6	487.3	506.5	510.5	
MROD #2															
Measurement count: 1	Ex: 538	Em: 590	Scaling Factor : 1/1												
Temp(°C)	25.7	1	1	2	3	4	5	6	7	8	9	10	11	12	
							2.588	12.08	12.27	11.92	2.762	21.13	20.86	23.07	
				2.177	14.53	98.95	2.654	20.63	19.99	20.71	2.796	27.1	28.68	29.74	
				2.075	14.46	99.21	2.586	19.7	20.13	18.74	2.806	20.88	21.42	22.4	
				2.109	14.17	99.43	2.716	28	28.69	26.28	2.785	21.54	21.78	22.58	
				8.2	50.42	134.9	2.722	21.8	21.04	19.6	2.836	20.96	21.81	23.64	
				8.451	50.94	137	2.784	22.59	21.97	20.66	2.717	18.78	19.25	21	
				8.784	50.93	135.9	2.665	17.18	17.51	16.56	2.314	2.108	2.05	2.268	
							2.726	25.81	26.27	24.2	2.514	2.051	2.138	2.096	
Protein #2															
Measurement count: 1	Ex: 355	Em: 460	Scaling Factor : 1/1												
			1	2	3	4	5	6	7	8	9	10	11	12	
							346.2	384.9	392.8	386.3	497.8	511.2	510.7	511.1	
				121.4	241.9	334.1	411.5	438.9	436.9	440.1	482.6	517.4	522.7	519.1	
				122.4	245.1	338.5	518.2	512.8	537	539	530.1	545.1	567.2	558.4	
				122	241.9	339.5	513.5	531.9	535.1	518.6	520.3	563	555.1	545.1	
				190.4	296.5	568.3	498.3	515.3	512.3	507.3	501.4	527.1	521.9	533.2	
				181.4	297.5	559.7	602.2	618.2	608.3	620.7	519	520.3	519.2	528.6	
				184.2	295.6	555.4	589	532.6	566.4	554.5	4.312	121.4	126.4	126.4	
							482.9	537.9	537	525.5	4.339	127.8	126	129.9	

## Data &amp; IDs

**MROD PLATE 1****Set 1: MROD Fluorescence Readings**

	1	2	3	4	5	6	7	8	9	10	11	12
A					2.685	28.32	29.91	30.89	2.709	26.03	26.06	29.3
B		1.919	14.33	98.25	2.577	38.33	36.98	35.74	2.647	29.1	29.12	33.74
C		1.858	14.32	98.43	2.474	23.78	26.03	25.2	2.857	30.21	31.42	34.44
D		1.919	14.19	98.08	2.566	21.01	22.67	21.73	2.685	22.71	26.11	26.32
E		8.182	50.04	132.9	2.517	20.53	21.18	20.92	2.656	20.15	20.43	22.14
F		8.382	49.92	133.2	2.712	29.23	32.11	26.92	2.637	16.55	17.38	19.07
G		8.466	49.94	133.8	2.635	27.42	27.15	26.35	2.736	21.35	21.83	24.51
H					2.666	27.24	22.91	26.04	2.55	24.17	26.68	29.27

**Set 2: Protein Fluorescence Readings**

	1	2	3	4	5	6	7	8	9	10	11	12
A					603.1	580.2	603.1	586.9	448.5	492.8	483.3	493.1
B		117.7	244.1	333.3	460.4	474.8	488.8	483.8	421.2	436.8	457.1	458.4
C		119.5	248.6	333.3	570.6	595.1	584.8	551	493.8	517.1	524	545.2
D		117.6	246	334.1	482.9	505.4	508.2	504.7	422.1	486.5	495	488.9
E		177.9	302.7	550.3	509.7	509.3	513.4	488.8	432.6	460	475.2	477.7
F		179.7	299.8	547.6	476.3	525.3	542.8	503.8	318.9	442.6	446.9	443.3
G		179.3	301	559.3	387.2	395.1	392.5	382.2	407.8	436.9	451.1	446.8
H					450.4	490.9	459.2	463.7	476.6	487.3	506.5	510.5

## Data & IDs

[illegible]

## Data &amp; IDs

**MROD PLATE 2****Set 1: MROD Fluorescence Readings**

	1	2	3	4	5	6	7	8	9	10	11	12
A					2.588	12.08	12.27	11.92	2.762	21.13	20.86	23.07
B		2.177	14.53	98.95	2.654	20.63	19.99	20.71	2.796	27.1	28.68	29.74
C		2.075	14.46	99.21	2.586	19.7	20.13	18.74	2.806	20.88	21.42	22.4
D		2.109	14.17	99.43	2.716	28	28.69	26.28	2.785	21.54	21.78	22.58
E		8.2	50.42	134.9	2.722	21.8	21.04	19.6	2.836	20.96	21.81	23.64
F		8.451	50.94	137	2.784	22.59	21.97	20.66	2.717	18.78	19.25	21
G		8.784	50.93	135.9	2.665	17.18	17.51	16.56				
H					2.726	25.81	26.27	24.2				

**Set 2: Protein Fluorescence Readings**

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	0	346.2	384.9	392.8	386.3	497.8	511.2	510.7	511.1
B	0	121.4	241.9	334.1	411.5	438.9	436.9	440.1	482.6	517.4	522.7	519.1
C	0	122.4	245.1	338.5	518.2	512.8	537	539	530.1	545.1	567.2	558.4
D	0	122	241.9	339.5	513.5	531.9	535.1	518.6	520.3	563	555.1	545.1
E	0	190.4	296.5	568.3	498.3	515.3	512.3	507.3	501.4	527.1	521.9	533.2
F	0	181.4	297.5	559.7	602.2	618.2	608.3	620.7	519	520.3	519.2	528.6
G	0	184.2	295.6	555.4	589	532.6	566.4	554.5				
H	0	0	0	0	482.9	537.9	537	525.5				

## Data & IDs

Cells	Entrix	Exponent	
	Sample ID	Group	Sample
A5-8	S38	Gp-2	38
B5-8	S39	Gp-4	39
C5-8	S40	Gp-2	40
D5-8	S41	Gp-6	41
E5-8	S42	Gp-6	42
F5-8	S43	Gp-4	43
G5-8	S44	Gp-1	44
H5-8	S46	Gp-1	46
A9-12	S47	Gp-3	47
B9-12	S48	Gp-6	48
C9-12	S50	Gp-3	50
D9-12	S51	Gp-6	51
E9-12	S53	Gp-4	53
F9-12	S54	Gp-2	54
G9-12			
H9-12			

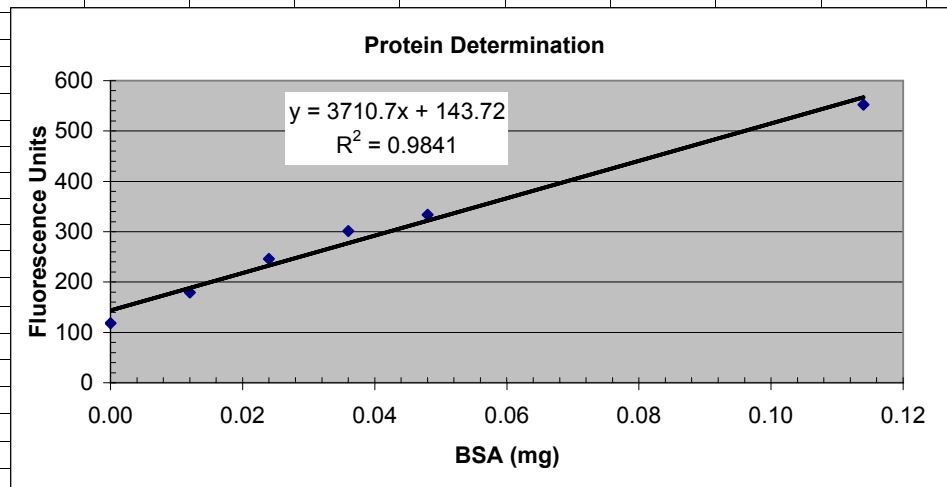
MROD#1 Analysis

<b>Samples:</b>	Liver Microsomes Processed on 3/6 to 3/8, 2006											
<b>Analysis:</b>	MROD analyses conducted on 03-21-2006											
<b>Plate #1</b>												
<b>Set 1: EROD Fluorescence Readings</b>												
	1	2	3	4	5	6	7	8	9	10	11	12
A				0.0	2.69	28.3	29.9	30.9	2.7	26.0	26.1	29.3
B		1.9	14.3	98.3	2.58	38.3	37.0	35.7	2.6	29.1	29.1	33.7
C		1.9	14.3	98.4	2.47	23.8	26.0	25.2	2.9	30.2	31.4	34.4
D		1.9	14.2	98.1	2.57	21.0	22.7	21.7	2.7	22.7	26.1	26.3
E		8.2	50.0	132.9	2.52	20.5	21.2	20.9	2.7	20.2	20.4	22.1
F		8.4	49.9	133.2	2.71	29.2	32.1	26.9	2.6	16.6	17.4	19.1
G		8.5	49.9	133.8	2.64	27.4	27.2	26.4	2.7	21.4	21.8	24.5
H					2.67	27.2	22.9	26.0	2.6	24.2	26.7	29.3
<b>Set 2: Protein Fluorescence Readings</b>												
	1	2	3	4	5	6	7	8	9	10	11	12
A					603	580	603	587	449	493	483	493
B		118	244	333	460	475	489	484	421	437	457	458
C		120	249	333	571	595	585	551	494	517	524	545
D		118	246	334	483	505	508	505	422	487	495	489
E		178	303	550	510	509	513	489	433	460	475	478
F		180	300	548	476	525	543	504	319	443	447	443
G		179	301	559	387	395	393	382	408	437	451	447
H					450	491	459	464	477	487	507	511

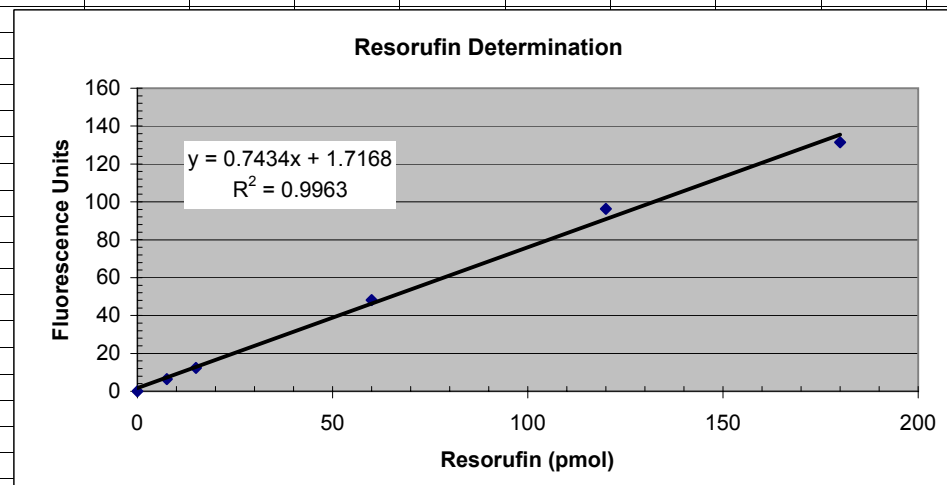


**Protein Determination**

BSA (mg)	Mean Fluor.
0.00	118
0.012	179
0.024	246
0.036	301
0.048	334
0.114	552

**Resorufin Determination**

Resorufin (pmol)	Mean Fluor.	Adj. Fluor.
0	1.90	0
7.5	8.3	6.4
15	14.3	12.4
60	50.0	48.1
120	98.3	96.4
180	133.3	131.4



## MROD#1 Analysis

[illegible]

## MROD#1 Analysis

MROD Activity (pmmol/min/mg)												
	1	2	3	4	5	6	7	8	9	10	11	12
A						30.4	30.6	32.9		34.8	35.8	39.3
B						55.2	51.0	49.9		46.6	43.6	50.8
C						24.4	27.5	28.8		38.1	39.0	40.7
D						26.6	28.7	27.7		30.6	34.7	35.6
E						25.7	26.3	27.8		29.1	28.2	30.5
F						36.0	38.0	34.9		24.8	25.8	28.9
G						51.0	51.0	51.6		33.4	32.7	37.5
H						36.7	33.5	37.9		32.6	34.3	37.5
Assay Time:		10 min										
MROD Activity (pmmol/min/mg)												
Cells	Sample ID	Blank	Rep 1	Raw Rep 2	Rep 3	Rep 1	Adjusted Rep 2	Rep 3	Mean	SD	Statistics CV (%)	
A5-8	S14	0.0	30.4	30.6	32.9	30.4	30.6	32.9	31.3	1.3	4.3	
B5-8	S15	0.0	55.2	51.0	49.9	55.2	51.0	49.9	52.0	2.78	5.3	
C5-8	S17	0.0	24.4	27.5	28.8	24.4	27.5	28.8	26.9	2.26	8.4	
D5-8	S18	0.0	26.6	28.7	27.7	26.6	28.7	27.7	27.7	1.03	3.7	
E5-8	S20	0.0	25.7	26.3	27.8	25.7	26.3	27.8	26.6	1.08	4.1	
F5-8	S22	0.0	36.0	38.0	34.9	36.0	38.0	34.9	36.3	1.56	4.3	
G5-8	S23	0.0	51.0	51.0	51.6	51.0	51.0	51.6	51.2	0.30	0.6	
H5-8	S24	0.0	36.7	33.5	37.9	36.7	33.5	37.9	36.1	2.27	6.3	
A9-12	S26	0.0	34.8	35.8	39.3	34.8	35.8	39.3	36.6	2.40	6.6	
B9-12	S29	0.0	46.6	43.6	50.8	46.6	43.6	50.8	47.0	3.59	7.6	
C9-12	S27	0.0	38.1	39.0	40.7	38.1	39.0	40.7	39.3	1.32	3.4	
D9-12	S30	0.0	30.6	34.7	35.6	30.6	34.7	35.6	33.6	2.67	7.9	
E9-12	S32	0.0	29.1	28.2	30.5	29.1	28.2	30.5	29.3	1.18	4.0	
F9-12	S35	0.0	24.8	25.8	28.9	24.8	25.8	28.9	26.5	2.16	8.1	
G9-12	S36	0.0	33.4	32.7	37.5	33.4	32.7	37.5	34.5	2.62	7.6	
H9-12	S37	0.0	32.6	34.3	37.5	32.6	34.3	37.5	34.8	2.47	7.1	
Sample Identifications (IDs) can be found in Laboratory Book (Dow#1)												

## MROD#2 Analysis

**Samples:** Liver Microsomes Processed on 3/6 to 3/8, 2006  
**Analysis:** MROD analyses conducted on 03-21-2006  
**Plate # 2**

### Set 1: MROD Fluorescence Readings

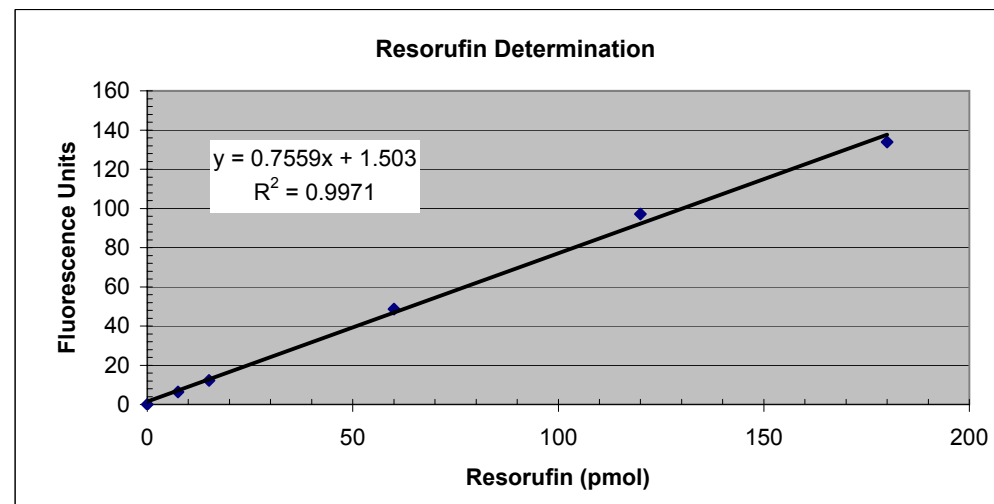
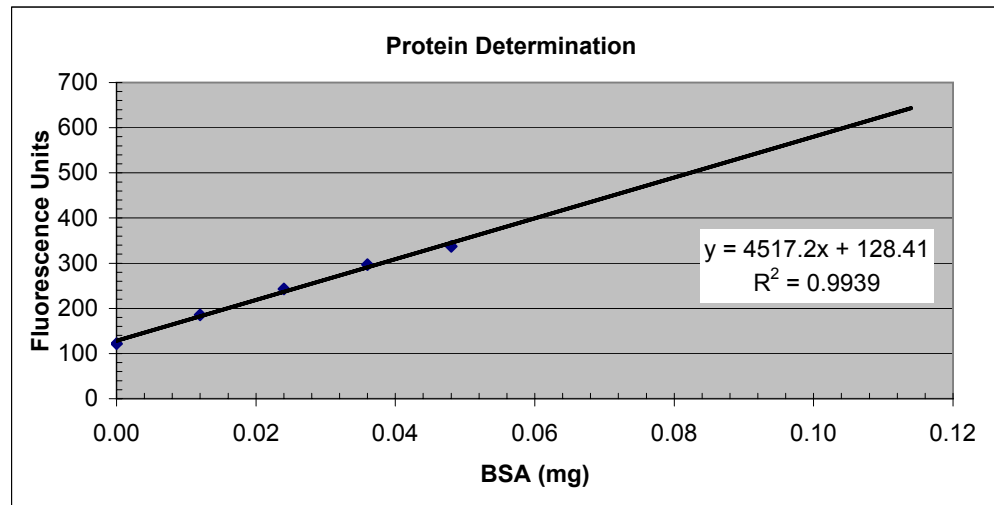
	1	2	3	4	5	6	7	8	9	10	11	12
A					2.59	12.08	12.27	11.92	2.76	21.13	20.86	23.07
B		2.18	14.53	98.95	2.65	20.63	19.99	20.71	2.80	27.10	28.68	29.74
C		2.08	14.46	99.21	2.59	19.70	20.13	18.74	2.81	20.88	21.42	22.40
D		2.11	14.17	99.43	2.72	28.00	28.69	26.28	2.79	21.54	21.78	22.58
E		8.20	50.42	134.90	2.72	21.80	21.04	19.60	2.84	20.96	21.81	23.64
F		8.45	50.94	137.00	2.78	22.59	21.97	20.66	2.72	18.78	19.25	21.00
G		8.78	50.93	135.90	2.67	17.18	17.51	16.56				
H					2.73	25.81	26.27	24.20				


**Protein Determination**

BSA (mg)	Mean Fluor.
0.00	122
0.012	185
0.024	243
0.036	297
0.048	337
0.114	561

**Resorufin Determination**

Resorufin (pmol)	Mean Fluor.	Adj. Fluor.
0	2.12	0
7.5	8.5	6.4
15	14.4	12.3
60	50.8	48.6
120	99.2	97.1
180	135.9	133.8



MROD#2 Analysis

**Set 1: Resorufin Content (pmol)**

	1	2	3	4	5	6	7	8	9	10	11	12
A					1.4	14.0	14.2	13.8	1.7	26.0	25.6	28.5
B					1.5	25.3	24.5	25.4	1.7	33.9	36.0	37.4
C					1.4	24.1	24.6	22.8	1.7	25.6	26.3	27.6
D					1.6	35.1	36.0	32.8	1.7	26.5	26.8	27.9
E					1.6	26.9	25.8	23.9	1.8	25.7	26.9	29.3
F					1.7	27.9	27.1	25.3	1.6	22.9	23.5	25.8
G					1.5	20.7	21.2	19.9				
H					1.6	32.2	32.8	30.0				

$$y = 1.7073x + 30.29$$

$$X = 0.7559$$

$$\text{Intercept} = 1.503$$

**Set 2: Protein Concentration (mg)**

	1	2	3	4	5	6	7	8	9	10	11	12
A					0.048	0.057	0.059	0.057	0.082	0.085	0.085	0.085
B					0.063	0.069	0.068	0.069	0.078	0.086	0.087	0.086
C					0.086	0.085	0.090	0.091	0.089	0.092	0.097	0.095
D					0.085	0.089	0.090	0.086	0.087	0.096	0.094	0.092
E					0.082	0.086	0.085	0.084	0.083	0.088	0.087	0.090
F					0.105	0.108	0.106	0.109	0.086	0.087	0.087	0.089
G					0.102	0.089	0.097	0.094				
H					0.078	0.091	0.090	0.088				

$$y = 20735x + 194.95$$

$$X = 4517.2$$

$$\text{Intercept} = 128.4$$

Not used

MROD#2 Analysis

**MROD Activity (pmmol/min/mg)**

	1	2	3	4	5	6	7	8	9	10	11	12
A						24.6	24.3	24.1		30.6	30.3	33.7
B						36.8	35.8	36.8		39.3	41.2	43.2
C						28.3	27.2	25.1		27.8	27.1	29.0
D						39.2	39.9	37.9		27.6	28.4	30.2
E						31.4	30.4	28.5		29.2	30.8	32.7
F						25.7	25.5	23.3		26.3	27.1	29.1
G						23.2	21.8	21.1				
H						35.5	36.2	34.2				

**Assay Time:** 10 min

**MROD Activity (pmmol/min/mg)**

Cells	Sample ID	Blank	Raw			Adjusted			Statistics		
			Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Mean	SD	CV (%)
A5-8	S38	0.0	24.6	24.3	24.1	24.6	24.3	24.1	24.4	0.3	1.0
B5-8	S39	0.0	36.8	35.8	36.8	36.8	35.8	36.8	36.5	0.58	1.6
C5-8	S40	0.0	28.3	27.2	25.1	28.3	27.2	25.1	26.9	1.63	6.1
D5-8	S41	0.0	39.2	39.9	37.9	39.2	39.9	37.9	39.0	1.02	2.6
E5-8	S42	0.0	31.4	30.4	28.5	31.4	30.4	28.5	30.1	1.43	4.7
F5-8	S43	0.0	25.7	25.5	23.3	25.7	25.5	23.3	24.8	1.36	5.5
G5-8	S44	0.0	23.2	21.8	21.1	23.2	21.8	21.1	22.0	1.05	4.7
H5-8	S46	0.0	35.5	36.2	34.2	35.5	36.2	34.2	35.3	1.05	3.0
A9-12	S47	0.0	30.6	30.3	33.7	30.6	30.3	33.7	31.5	1.87	5.9
B9-12	S48	0.0	39.3	41.2	43.2	39.3	41.2	43.2	41.2	1.93	4.7
C9-12	S50	0.0	27.8	27.1	29.0	27.8	27.1	29.0	28.0	0.97	3.5
D9-12	S51	0.0	27.6	28.4	30.2	27.6	28.4	30.2	28.7	1.37	4.8
E9-12	S53	0.0	29.2	30.8	32.7	29.2	30.8	32.7	30.9	1.76	5.7
F9-12	S54	0.0	26.3	27.1	29.1	26.3	27.1	29.1	27.5	1.43	5.2
G9-12											
H9-12											

Sample Identifications (IDs) can be found in Laboratory Book (Dow#1)

# Summary

## Summary of MROD Results

Entrix	Exponent		Statistics			Group Statistics	
Sample ID	Group	Sample	Mean	Stdev	CV (%)	Mean	Stdev
S17	Gp-1	17	26.9	2.26	8.18	25.7	2.2
S18	Gp-1	18	27.7	1.03	3.76		
S20	Gp-1	20	26.6	1.08	3.87		
S44	Gp-1	44	22.0	1.05	4.89		
S46	Gp-1	46	25.3	1.05	2.89		
S32	Gp-2	32	29.3	1.18	4.09	26.9	1.8
S35	Gp-2	35	26.5	2.16	8.14		
S38	Gp-2	38	24.4	0.30	1.09		
S40	Gp-2	40	26.9	1.63	6.22		
S54	Gp-2	54	27.5	1.43	5.12		
S22	Gp-3	22	36.3	1.56	4.56	33.3	3.6
S24	Gp-3	24	36.1	2.27	6.42		
S37	Gp-3	37	34.8	2.47	7.26		
S47	Gp-3	47	31.5	1.87	5.94		
S50	Gp-3	50	28.0	0.97	3.50		
S14	Gp-4	14	31.3	1.30	4.29	34.9	10.0
S23	Gp-4	23	51.2	0.30	0.38		
S39	Gp-4	39	36.5	0.58	1.56		
S43	Gp-4	43	24.8	1.36	5.52		
S53	Gp-4	53	30.9	1.76	5.65		
S15	Gp-5	15	52.0	2.78	5.24	41.9	7.4
S26	Gp-5	26	36.6	2.40	6.58		
S29	Gp-5	29	47.0	3.59	7.68		
S27	Gp-5	27	39.3	1.32	3.54		
S36	Gp-5	36	34.5	2.62	7.61		
S30	Gp-6	30	33.6	2.67	7.97	34.5	5.5
S41	Gp-6	41	39.0	1.02	2.50		
S42	Gp-6	42	30.1	1.43	4.70		
S48	Gp-6	48	41.2	1.93	4.68		
S51	Gp-6	51	28.7	1.37	4.86		



## **Appendix D**

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### **Detailed Study Data**

**Table D-1. Rat feed intake during the follow-up study**

Date: Study Day:	2-Day Feed Intake (g)															Total Feed Intake (g)
	25-Jan 2	27-Jan 4	29-Jan 6	31-Jan 8	2-Feb 10	4-Feb 12	6-Feb 14	8-Feb 16	10-Feb 18	12-Feb 20	14-Feb 22	16-Feb 24	18-Feb 26	20-Feb 28	22-Feb 30	
Group 1: Feed Control																
17	33.15	30.83	31.56	28.46	36.92	32.01	32.79	36.88	28.96	36.88	33.44	32.54	36.44	24.59	32.99	488.44
18	25.49	36.99	31.63	33.73	29.82	34.51	39.92	32.49	37.33	36.96	32.91	41.51	33.94	36.94	33.09	517.26
20	26.63	35.64	29.09	34.60	32.61	31.26	38.22	29.80	35.88	35.40	31.60	35.67	29.34	35.21	33.58	494.53
44	31.39	30.38	38.08	37.56	32.82	41.00	33.42	41.98	44.06	38.54	43.50	32.98	41.99	34.74	32.11	554.55
46	24.34	22.86	29.82	24.33	26.05	26.02	33.85	31.72	30.24	36.67	31.30	31.63	33.18	28.34	33.25	443.60
Mean:	28.20	31.34	32.04	31.74	31.64	32.96	35.64	34.57	35.29	36.89	34.55	34.87	34.98	31.96	33.00	499.68
Group 2: Oil Control																
19 <sup>a</sup>	27.82	31.31	26.39	28.12	26.73	32.74	33.07	32.36	27.96	27.66	28.31	29.68	29.64	30.16	28.11	--
25 <sup>b</sup>	25.16	30.08	22.66	0.27	--	--	--	--	--	--	--	--	--	--	--	--
32	25.37	22.28	24.43	21.42	20.59	25.80	19.53	26.71	22.94	21.00	26.79	19.45	24.78	25.96	22.15	349.20
35	29.46	21.51	24.65	26.02	23.18	33.30	31.89	32.83	24.02	27.60	27.00	18.33	24.18	23.60	26.13	393.70
38	26.84	27.75	33.66	32.42	24.00	31.30	32.76	28.10	30.57	25.35	28.12	28.06	28.81	32.74	20.49	430.97
40	22.83	18.24	27.08	21.35	26.06	21.21	26.37	18.93	23.67	18.13	28.33	20.20	24.32	21.46	24.87	343.05
54	26.31	30.31	33.04	24.98	27.20	29.10	33.34	24.27	34.35	33.02	31.66	31.84	30.50	32.95	30.33	453.20
Mean:	26.16	24.02	28.57	25.24	24.21	28.14	28.78	26.17	27.11	25.02	28.38	23.58	26.52	27.34	24.79	394.02
Group 3: Oil Reference 0.2X																
22	22.83	24.61	30.70	33.54	32.37	30.63	21.18	23.39	19.59	23.77	18.15	29.42	24.67	25.48	28.29	388.62
24	25.52	29.38	27.17	27.35	26.12	32.34	32.21	25.57	32.46	27.22	27.93	31.61	23.60	25.41	23.44	417.33
37	27.49	29.33	26.62	28.45	20.82	31.32	24.49	28.35	28.51	22.78	34.85	24.97	30.60	28.29	25.29	412.16
45 <sup>a</sup>	24.45	25.88	24.71	27.54	20.68	26.31	28.10	21.13	28.02	23.89	22.42	30.09	25.05	16.75	0.14	--
47	27.07	27.41	26.49	25.56	25.43	23.93	29.70	27.38	24.19	30.43	27.75	29.28	34.02	26.45	32.18	417.27
49 <sup>a</sup>	24.82	26.60	29.43	29.11	28.41	30.94	24.33	34.70	27.20	26.80	34.51	22.91	37.50	30.73	25.43	--
50	26.94	24.98	29.14	24.24	24.07	25.17	25.35	27.28	27.21	21.16	28.82	23.66	31.01	24.83	24.24	388.10
Mean:	25.97	27.14	28.02	27.83	25.76	28.68	26.59	26.39	26.39	25.07	27.50	27.79	28.78	26.09	26.69	404.70
Group 4: Oil Reference 0.5X																
14	25.54	25.79	17.01	27.77	23.07	26.21	20.33	20.39	24.20	6.33	25.34	30.74	36.95	27.24	34.09	371.00
21 <sup>a</sup>	26.10	32.94	26.28	28.88	23.76	28.58	30.85	20.26	27.55	31.91	25.51	30.04	23.16	30.95	23.42	--
23	22.67	26.65	32.43	22.06	26.97	24.13	29.45	29.86	28.13	29.93	24.49	30.27	30.49	30.64	29.96	418.13
33 <sup>a</sup>	26.29	30.14	26.90	22.27	22.79	23.81	26.28	28.10	31.53	23.10	32.35	36.04	35.44	43.59	38.59	--
39	23.39	25.04	33.88	25.66	33.19	31.68	31.78	34.97	28.22	30.35	32.65	28.82	34.80	28.29	28.81	451.53
43	26.86	30.62	33.36	34.20	34.76	30.15	27.89	21.33	33.42	24.45	28.00	32.51	25.43	31.64	26.77	441.39
53	25.80	31.59	30.91	36.63	31.09	34.70	35.53	24.74	31.20	31.36	27.73	31.13	26.45	35.36	32.26	466.48
Mean:	24.85	27.94	29.52	29.26	29.82	29.37	29.00	26.26	29.03	24.48	27.64	30.69	30.82	30.63	30.38	429.71

Table D-1. (cont.)

Date: Study Day:	2-Day Feed Intake (g)															Total Feed Intake (g)
	25-Jan 2	27-Jan 4	29-Jan 6	31-Jan 8	2-Feb 10	4-Feb 12	6-Feb 14	8-Feb 16	10-Feb 18	12-Feb 20	14-Feb 22	16-Feb 24	18-Feb 26	20-Feb 28	22-Feb 30	
Group 5: Oil Reference 0.8X																
15	22.41	25.05	27.71	22.33	28.54	23.84	30.95	26.26	30.06	32.11	24.97	30.13	29.01	28.33	31.18	412.88
26	21.25	23.87	26.33	21.88	24.62	25.72	32.05	19.55	32.84	35.16	28.87	32.60	26.05	31.12	22.04	403.95
27	21.73	28.41	24.12	23.40	21.49	28.94	23.46	25.40	21.20	26.75	26.33	24.17	27.95	26.31	24.21	373.87
28 <sup>a</sup>	22.44	20.27	27.24	21.21	27.04	20.19	27.06	24.04	28.72	24.77	23.04	28.70	22.12	27.79	24.99	--
29	27.75	37.38	39.79	33.81	37.71	34.64	31.63	37.23	22.03	39.07	36.35	31.50	38.33	31.14	36.14	514.50
36	18.65	28.33	31.84	29.11	31.34	30.04	34.74	30.63	34.21	22.16	24.16	35.36	32.47	32.77	29.38	445.19
52 <sup>b</sup>	24.71	25.12	27.10	27.57	12.46	--	--	--	--	--	--	--	--	--	--	--
Mean:	22.36	28.61	29.96	26.11	28.74	28.64	30.57	27.81	28.07	31.05	28.14	30.75	30.76	29.93	28.59	430.08
Group 6: Soil																
30	39.40	37.11	39.86	35.91	36.62	35.29	38.75	36.46	40.31	34.79	40.02	33.89	37.63	37.29	32.93	556.26
41	33.81	40.92	38.26	33.89	40.31	33.20	40.64	43.86	38.10	45.21	40.19	40.08	44.26	38.48	38.81	590.02
42	33.01	32.66	34.83	36.54	33.92	41.92	37.25	35.85	40.37	32.62	38.43	36.49	35.65	37.54	30.71	537.79
48	28.32	37.65	27.64	37.53	31.61	32.30	38.34	27.13	38.67	29.00	32.21	35.63	28.42	37.24	32.07	493.76
51	26.54	38.20	32.34	37.60	40.04	33.70	41.14	32.05	39.44	42.25	30.72	37.28	31.70	38.21	33.34	534.55
Mean:	32.22	37.31	34.59	36.29	36.50	35.28	39.22	35.07	39.38	36.77	36.31	36.67	35.53	37.75	33.57	542.48

<sup>a</sup> To allow for gavage-related mortality, seven rats, rather than five, were included in each of the corn oil gavage groups during the compound administration phase of the study.

This rat was randomly selected to be excluded from the final group used for tissue collection, and feed intake values for this animal are not included in the group means.

<sup>b</sup> This rat was euthanized before the end of the study. Feed intake values for this animal are not included in the group means.

**Table D-2. Rat body weights during the follow-up study**

Date: Study Day:	Body Weight (g)						Mean
	18-Jan -5	24-Jan 1	30-Jan 7	7-Feb 15	14-Feb 22	22-Feb 30	Body Weight (g)
<b>Group 1: Feed Control</b>							
17	260.00	271.10	266.43	276.55	267.87	275.73	271.54
18	266.10	271.40	273.42	282.37	282.77	285.47	279.09
20	262.10	264.59	268.23	273.94	275.20	282.15	272.82
44	266.50	272.31	278.60	288.44	290.69	294.25	284.86
46	287.80	294.23	280.61	281.65	267.48	274.20	279.63
Mean:	268.50	274.73	273.46	280.59	276.80	282.36	277.59
<b>Group 2: Oil Control</b>							
19 <sup>b</sup>	256.30	280.67	260.09	267.71	268.25	--	--
25 <sup>c</sup>	272.40	278.90	264.74	--	--	--	--
32	263.50	268.10	267.93	260.41	260.22	264.95	264.32
35	255.70	247.44	257.95	275.88	263.35	264.98	261.92
38	279.90	286.65	289.17	289.97	286.76	285.30	287.57
40	255.90	257.43	256.08	254.48	255.45	255.49	255.79
54	260.00	267.67	268.07	271.78	279.51	280.96	273.60
Mean:	263.00	265.46	267.84	270.50	269.06	270.34	268.64
<b>Group 3: Oil Reference 0.2X</b>							
22	249.50	256.36	264.69	266.17	261.76	267.10	263.22
24	282.90	289.14	285.66	291.59	294.02	287.66	289.61
37	285.90	288.56	291.09	287.29	288.03	286.63	288.32
45 <sup>b</sup>	263.20	264.77	267.87	269.82	268.94	--	--
47	267.90	278.37	274.31	279.59	280.98	294.78	281.61
49 <sup>b</sup>	276.70	269.59	274.95	275.94	278.29	--	--
50	263.60	273.71	277.26	278.15	275.98	279.33	276.89
Mean:	269.96	277.23	278.60	280.56	280.15	283.10	279.93
<b>Group 4: Oil Reference 0.5X</b>							
14	285.10	288.96	278.02	282.48	269.93	294.77	282.83
21 <sup>b</sup>	269.30	279.29	276.86	277.89	281.79	--	--
23	279.40	292.15	288.49	292.02	290.48	296.23	291.87
33 <sup>b</sup>	257.50	264.16	260.28	251.14	262.09	--	--
39	277.70	279.84	282.00	293.87	289.06	298.82	288.72
43	277.50	280.31	286.13	280.38	280.95	277.93	281.14
53	267.40	264.80	275.43	281.09	282.84	291.15	279.06
Mean:	277.42	281.21	282.01	285.97	282.65	291.78	284.72
<b>Group 5: Oil Reference 0.8X</b>							
15	250.60	257.91	257.27	264.59	268.47	276.11	264.87
26	275.30	271.07	265.84	262.17	266.11	261.30	265.30
27	262.70	265.48	260.25	258.62	260.19	262.98	261.50
28 <sup>b</sup>	262.50	257.73	250.94	252.56	252.84	--	--
29	269.70	264.27	273.47	282.36	279.61	280.73	276.09
36	254.90	251.64	255.84	272.50	276.74	281.86	267.72
52 <sup>c</sup>	257.90	261.84	256.02	--	--	--	--
Mean:	262.64	262.07	262.53	268.05	270.22	272.60	267.10

**Table D-2. (cont.)**

Date Study Day	Body Weight (g)						Mean Body Weight (g)
	18-Jan -5	24-Jan 1	30-Jan 7	7-Feb 15	14-Feb 22	22-Feb 30	
Group 6: Soil							
30	265.30	279.34	282.45	292.53	296.60	294.65	289.11
41	265.70	274.73	282.27	291.34	286.97	288.25	284.71
42	261.00	259.19	265.70	276.95	283.14	280.78	273.15
48	259.10	257.16	263.06	258.96	259.30	257.50	259.20
51	282.20	256.15	273.55	277.99	277.72	276.55	272.39
Mean:	266.66	265.31	273.41	279.55	280.75	279.55	275.71

<sup>a</sup> Mean of body weights from study days 1, 7, 15, 22, and 30.

<sup>b</sup> To allow for gavage-related mortality, seven rats, rather than five, were included in each of the corn oil gavage groups during the compound administration phase of the study. This rat was randomly selected to be excluded from the final group used for tissue collection, and feed intake values for this animal are not included in the group means.

<sup>c</sup> This rat was euthanized before the end of the study. Feed intake values for this animal are not included in the group means.

**Table D-3. Rat necropsy liver and fat sample weights**

Rat #	Liver Weight (g)	Abdominal Fat Sample Weight (g)
<b>Group 1: Feed Control</b>		
17	10.87	2.85
18	10.08	5.02
20	11.38	3.62
44	10.50	5.58
46	8.92	4.23
<b>Gp 1 Mean</b>	<b>10.35</b>	<b>4.26</b>
<b>Group 2: Oil Control</b>		
32	8.10	2.99
35	9.27	3.15
38	10.61	3.70
40	8.09	4.76
54	9.63	5.04
<b>Gp 2 Mean</b>	<b>9.14</b>	<b>3.93</b>
<b>Group 3: Oil Reference 0.2X</b>		
22	8.45	5.21
24	8.91	4.64
37	9.77	4.08
47	10.31	3.84
50	8.89	4.44
<b>Gp 3 Mean</b>	<b>9.27</b>	<b>4.44</b>
<b>Group 4: Oil Reference 0.5X</b>		
14	10.59	6.83
23	10.19	4.55
39	9.93	4.79
43	8.54	3.57
53	12.23	5.26
<b>Gp 4 Mean</b>	<b>10.30</b>	<b>5.00</b>
<b>Group 5: Oil Reference 0.8X</b>		
15	10.19	4.16
26	8.73	3.56
27	8.63	3.29
29	9.13	4.26
36	10.03	4.19
<b>Gp 5 Mean</b>	<b>9.34</b>	<b>3.89</b>
<b>Group 6: Soil</b>		
30	10.30	4.00
41	9.10	5.48
42	9.48	3.96
48	8.41	3.38
51	9.13	2.85
<b>Gp 6 Mean</b>	<b>9.28</b>	<b>3.93</b>

**Notes:**

Liver was weighed, EROD/MROD sample cut out, remainder wrapped in foil and placed on dry ice.

For fat samples, samplers tried to get 4–5 g from same areas on all rats. Fat samples were weighed, wrapped in foil, and placed on dry ice

**Table D-4. Tissue concentrations, doses, and RBA calculations for the rat follow-up study**

Tittabawassee River Soil (Group 6)													
Analyte	Soil/ Diet Mean Conc. (pg/g)	Rat IDs	Total Feed Intake (g)	Mean BW <sup>c</sup> (g)	Terminal BW (g)	Using Mean BW			Total Dose (pg)	Liver Weight (g)	Liver Conc. (pg/g)	Using Terminal BW	
						Total Dose[bw] <sup>a</sup> (pg/g)	Avg. Daily Dose[bw] <sup>a</sup> (pg/g)	Avg. Daily Dose[bw] <sup>a</sup> S.D.				Fat Weight Fraction (wa) (unitless)	Fat Weight (g)
2,3,7,8-TCDF	79.3	Grp 6 Mean	542.48	275.71	279.55	156	5.198	0.171	43,019		300		
1,2,3,7,8-PeCDF	49.5	Grp 6 Mean	542.48	275.71	279.55	97.4	3.245	0.107	26,853		230		
2,3,4,7,8-PeCDF	42.3	Grp 6 Mean	542.48	275.71	279.55	83.2	2.773	0.091	22,947		1,066		
1,2,3,4,7,8-HxCDF	33.4	Grp 6 Mean	542.48	275.71	279.55	65.7	2.189	0.072	18,119		575		
1,2,3,6,7,8-HxCDF	8.19	Grp 6 Mean	542.48	275.71	279.55	16.1	0.537	0.018	4,443		158		
2,3,7,8-TCDF	79.3	30	556.26	289.11	294.65	153	5.086		44,111	10.3	311	0.0753	22.18
1,2,3,7,8-PeCDF	49.5	30	556.26	289.11	294.65	95.2	3.175		27,535	10.3	238	0.0753	22.18
2,3,4,7,8-PeCDF	42.3	30	556.26	289.11	294.65	81.4	2.713		23,530	10.3	1,040	0.0753	22.18
1,2,3,4,7,8-HxCDF	33.4	30	556.26	289.11	294.65	64.3	2.142		18,579	10.3	554	0.0753	22.18
1,2,3,6,7,8-HxCDF	8.19	30	556.26	289.11	294.65	15.8	0.525		4,556	10.3	153	0.0753	22.18
2,3,7,8-TCDF	79.3	41	590.02	284.71	288.25	164	5.478		46,789	9.10	319	0.074	21.33
1,2,3,7,8-PeCDF	49.5	41	590.02	284.71	288.25	103	3.419		29,206	9.10	232	0.074	21.33
2,3,4,7,8-PeCDF	42.3	41	590.02	284.71	288.25	87.7	2.922		24,958	9.10	1,060	0.074	21.33
1,2,3,4,7,8-HxCDF	33.4	41	590.02	284.71	288.25	69.2	2.307		19,707	9.10	575	0.074	21.33
1,2,3,6,7,8-HxCDF	8.19	41	590.02	284.71	288.25	17.0	0.566		4,832	9.10	154	0.074	21.33
2,3,7,8-TCDF	79.3	42	537.79	273.15	280.78	156	5.204		42,647	9.48	258	0.0725	20.36
1,2,3,7,8-PeCDF	49.5	42	537.79	273.15	280.78	97.5	3.249		26,621	9.48	198	0.0725	20.36
2,3,4,7,8-PeCDF	42.3	42	537.79	273.15	280.78	83.3	2.776		22,749	9.48	1,000	0.0725	20.36
1,2,3,4,7,8-HxCDF	33.4	42	537.79	273.15	280.78	65.8	2.192		17,962	9.48	544	0.0725	20.36
1,2,3,6,7,8-HxCDF	8.19	42	537.79	273.15	280.78	16.1	0.537		4,405	9.48	151	0.0725	20.36
2,3,7,8-TCDF	79.3	48	493.76	259.20	257.50	151	5.035		39,155	8.41	325	0.0679	17.48
1,2,3,7,8-PeCDF	49.5	48	493.76	259.20	257.50	94.3	3.143		24,441	8.41	253	0.0679	17.48
2,3,4,7,8-PeCDF	42.3	48	493.76	259.20	257.50	80.6	2.686		20,886	8.41	1,180	0.0679	17.48
1,2,3,4,7,8-HxCDF	33.4	48	493.76	259.20	257.50	63.6	2.121		16,492	8.41	635	0.0679	17.48
1,2,3,6,7,8-HxCDF	8.19	48	493.76	259.20	257.50	15.6	0.520		4,044	8.41	178	0.0679	17.48
2,3,7,8-TCDF	79.3	51	534.55	272.39	276.55	156	5.187		42,390	9.13	287	0.0717	19.82
1,2,3,7,8-PeCDF	49.5	51	534.55	272.39	276.55	97.1	3.238		26,460	9.13	227	0.0717	19.82
2,3,4,7,8-PeCDF	42.3	51	534.55	272.39	276.55	83.0	2.767		22,611	9.13	1,050	0.0717	19.82
1,2,3,4,7,8-HxCDF	33.4	51	534.55	272.39	276.55	65.5	2.185		17,854	9.13	567	0.0717	19.82
1,2,3,6,7,8-HxCDF	8.19	51	534.55	272.39	276.55	16.1	0.536		4,378	9.13	156	0.0717	19.82

Table D-4. (cont.)

Analyte	Tittabawassee River Soil (Group 6)										Oil Reference 0.2X (Group 3)			
	Fat Conc. (pg/g)	Fraction Retained in Liver FR <sub>liver</sub> (unitless)	FR <sub>liver</sub> S.D.	Fraction Retained in Fat FR <sub>fat</sub> (unitless)	FR <sub>fat</sub> S.D.	Fraction Retained Liver+Fat FR <sub>sum</sub> (unitless)	FR <sub>sum</sub> S.D.	WHO TEF (unitless)	Liver TEQ (pg/g)	Liver TEQ SD	Oil Reference 0.2X Mean Conc. (ng/mL)	Group 3 Rat IDs	Total Gavage Volume (mL)	Mean BW <sup>c</sup> (g)
2,3,7,8-TCDF	138	0.065	0.006	0.065	0.007	0.130	0.012	0.1	30.0	2.76	0.268	Grp 3 Mean	30	279.93
1,2,3,7,8-PeCDF	58.4	0.079	0.008	0.044	0.003	0.123	0.011	0.05	11.5	1.01	0.185	Grp 3 Mean	30	279.93
2,3,4,7,8-PeCDF	55.7	0.432	0.035	0.049	0.004	0.481	0.037	0.5	533	33.8	0.166	Grp 3 Mean	30	279.93
1,2,3,4,7,8-HxCDF	37.5	0.295	0.022	0.042	0.005	0.337	0.026	0.1	57.5	3.56	0.122	Grp 3 Mean	30	279.93
1,2,3,6,7,8-HxCDF	10.5	0.331	0.030	0.048	0.007	0.379	0.035	0.1	15.8	1.11	0.036	Grp 3 Mean	30	279.93
2,3,7,8-TCDF	149	0.073		0.075		0.148		0.1	31.1		0.268	22	30	263.22
1,2,3,7,8-PeCDF	62.0	0.089		0.050		0.139		0.05	11.9		0.185	22	30	263.22
2,3,4,7,8-PeCDF	57.7	0.455		0.054		0.510		0.5	520		0.166	22	30	263.22
1,2,3,4,7,8-HxCDF	39.8	0.307		0.048		0.355		0.1	55.4		0.122	22	30	263.22
1,2,3,6,7,8-HxCDF	10.8 J	0.346		0.053		0.398		0.1	15.3		0.036	22	30	263.22
2,3,7,8-TCDF	150	0.062		0.068		0.130		0.1	31.9		0.268	24	30	289.61
1,2,3,7,8-PeCDF	58.1	0.072		0.042		0.115		0.05	11.6		0.185	24	30	289.61
2,3,4,7,8-PeCDF	51.6	0.386		0.044		0.431		0.5	530		0.166	24	30	289.61
1,2,3,4,7,8-HxCDF	31.1	0.266		0.034		0.299		0.1	57.5		0.122	24	30	289.61
1,2,3,6,7,8-HxCDF	8.00 J	0.290		0.035		0.325		0.1	15.4		0.036	24	30	289.61
2,3,7,8-TCDF	126	0.057		0.060		0.118		0.1	25.8		0.268	37	30	288.32
1,2,3,7,8-PeCDF	56.5	0.071		0.043		0.114		0.05	9.90		0.185	37	30	288.32
2,3,4,7,8-PeCDF	54.7	0.417		0.049		0.466		0.5	500		0.166	37	30	288.32
1,2,3,4,7,8-HxCDF	38.3	0.287		0.043		0.331		0.1	54.4		0.122	37	30	288.32
1,2,3,6,7,8-HxCDF	11.0 J	0.325		0.051		0.376		0.1	15.1		0.036	37	30	288.32
2,3,7,8-TCDF	142	0.070		0.063		0.133		0.1	32.5		0.268	47	30	281.61
1,2,3,7,8-PeCDF	60.1	0.087		0.043		0.130		0.05	12.7		0.185	47	30	281.61
2,3,4,7,8-PeCDF	58.7	0.475		0.049		0.524		0.5	590		0.166	47	30	281.61
1,2,3,4,7,8-HxCDF	40.6	0.324		0.043		0.367		0.1	63.5		0.122	47	30	281.61
1,2,3,6,7,8-HxCDF	11.4 J	0.370		0.049		0.419		0.1	17.8		0.036	47	30	281.61
2,3,7,8-TCDF	123	0.062		0.058		0.119		0.1	28.7		0.268	50	30	276.89
1,2,3,7,8-PeCDF	55.4	0.078		0.042		0.120		0.05	11.4		0.185	50	30	276.89
2,3,4,7,8-PeCDF	56.0	0.424		0.049		0.473		0.5	525		0.166	50	30	276.89
1,2,3,4,7,8-HxCDF	37.8	0.290		0.042		0.332		0.1	56.7		0.122	50	30	276.89
1,2,3,6,7,8-HxCDF	11.5 J	0.325		0.052		0.377		0.1	15.6		0.036	50	30	276.89



Table D-4. (cont.)

Oil Reference 0.2X (Group 3)														
Analyte	Terminal BW (g)	Using Mean BW			Total Dose (pg)	Liver Weight (g)	Liver Conc. (pg/g)	Using Terminal BW			Fraction Retained in Liver FR <sub>liver</sub> (unitless)	FR <sub>liver</sub> S.D.	Fraction Retained in Fat FR <sub>fat</sub> (unitless)	FR <sub>fat</sub> S.D.
		Total Dose[bw] <sup>b</sup> (pg/g)	Avg. Daily Dose[bw] <sup>a</sup> (pg/g)	Avg. Daily Dose[bw] <sup>a</sup> S.D.				Fat Weight Fraction (wa) (unitless)	Fat Weight (g)	Fat Conc. (pg/g)				
2,3,7,8-TCDF	283.10	28.7	0.959	0.038	8,040		103			60.5	0.118	0.012	0.155	0.006
1,2,3,7,8-PeCDF	283.10	19.8	0.662	0.026	5,550		74.2			24.7	0.123	0.009	0.091	0.004
2,3,4,7,8-PeCDF	283.10	17.8	0.594	0.023	4,980		358			24.8	0.656	0.010	0.100	0.011
1,2,3,4,7,8-HxCDF	283.10	13.1	0.436	0.017	3,660		175			15.1	0.435	0.016	0.083	0.010
1,2,3,6,7,8-HxCDF	283.10	3.86	0.129	0.005	1,080		52.6			4.36	0.446	0.009	0.081	0.006
2,3,7,8-TCDF	267.10	30.5	1.018		8,040	8.45	122	0.0698	18.64	63.4	0.128		0.147	
1,2,3,7,8-PeCDF	267.10	21.1	0.703		5,550	8.45	86.8	0.0698	18.64	25.9	0.132		0.087	
2,3,4,7,8-PeCDF	267.10	18.9	0.631		4,980	8.45	394	0.0698	18.64	23.6	0.669		0.088	
1,2,3,4,7,8-HxCDF	267.10	13.9	0.463		3,660	8.45	195	0.0698	18.64	14.8	0.450		0.075	
1,2,3,6,7,8-HxCDF	267.10	4.10	0.137		1,080	8.45	58.4	0.0698	18.64	4.33 J	0.457		0.075	
2,3,7,8-TCDF	287.66	27.8	0.925		8,040	8.91	116	0.0739	21.25	58.9	0.129		0.156	
1,2,3,7,8-PeCDF	287.66	19.2	0.639		5,550	8.91	79.9	0.0739	21.25	22.9	0.128		0.088	
2,3,4,7,8-PeCDF	287.66	17.2	0.573		4,980	8.91	362	0.0739	21.25	21.9	0.648		0.093	
1,2,3,4,7,8-HxCDF	287.66	12.6	0.421		3,660	8.91	177	0.0739	21.25	13.0 J	0.431		0.075	
1,2,3,6,7,8-HxCDF	287.66	3.73	0.124		1,080	8.91	53.6	0.0739	21.25	4.03 J	0.442		0.079	
2,3,7,8-TCDF	286.63	27.9	0.930		8,040	9.77	99.1	0.0737	21.12	59.3	0.120		0.156	
1,2,3,7,8-PeCDF	286.63	19.2	0.642		5,550	9.77	70.1	0.0737	21.12	25.5	0.123		0.097	
2,3,4,7,8-PeCDF	286.63	17.3	0.576		4,980	9.77	351	0.0737	21.12	27.1	0.689		0.115	
1,2,3,4,7,8-HxCDF	286.63	12.7	0.423		3,660	9.77	174	0.0737	21.12	16.3	0.464		0.094	
1,2,3,6,7,8-HxCDF	286.63	3.75	0.125		1,080	9.77	51.3	0.0737	21.12	4.89 J	0.464		0.096	
2,3,7,8-TCDF	294.78	28.6	0.952		8,040	10.31	84.7	0.0753	22.20	56.9	0.109		0.157	
1,2,3,7,8-PeCDF	294.78	19.7	0.657		5,550	10.31	60.8	0.0753	22.20	23.3	0.113		0.093	
2,3,4,7,8-PeCDF	294.78	17.7	0.589		4,980	10.31	319	0.0753	22.20	24.9	0.660		0.111	
1,2,3,4,7,8-HxCDF	294.78	13.0	0.433		3,660	10.31	158	0.0753	22.20	15.9	0.445		0.096	
1,2,3,6,7,8-HxCDF	294.78	3.84	0.128		1,080	10.31	47	0.0753	22.20	4.34 J	0.449		0.089	
2,3,7,8-TCDF	279.33	29.0	0.968		8,040	8.89	95.1	0.0722	20.18	64.0	0.105		0.161	
1,2,3,7,8-PeCDF	279.33	20.0	0.668		5,550	8.89	73.2	0.0722	20.18	26.1	0.117		0.095	
2,3,4,7,8-PeCDF	279.33	18.0	0.600		4,980	8.89	363	0.0722	20.18	26.3	0.648		0.107	
1,2,3,4,7,8-HxCDF	279.33	13.2	0.441		3,660	8.89	171	0.0722	20.18	15.3	0.415		0.084	
1,2,3,6,7,8-HxCDF	279.33	3.90	0.130		1,080	8.89	52.9	0.0722	20.18	4.23 J	0.435		0.079	

Table D-4. (cont.)

Oil Reference 0.2X (Group 3)					Oil Reference 0.5X (Group 4)									
Analyte	Fraction Retained Liver+Fat		WHO	Liver	Oil Reference 0.5X Mean Conc.		Total Gavage Volume	Mean BW <sup>c</sup>	Terminal BW	Using Mean BW			Total Dose	Liver Weight
	FR <sub>sum</sub> (unitless)	FR <sub>sum</sub> S.D.	TEF (unitless)	TEQ (pg/g)	(ng/mL)	Group 4 Rat IDs	(mL)	(g)	(g)	Total Dose[bw] <sup>b</sup> (pg/g)	Avg. Daily Dose[bw] <sup>a</sup> (pg/g)	Avg. Daily Dose[bw] <sup>a</sup> S.D.	(pg)	(g)
2,3,7,8-TCDF	0.273	0.009	0.1	10.4	0.673	Grp 4 Mean	30	284.72	291.78	70.9	2.364	0.044	20,190	
1,2,3,7,8-PeCDF	0.213	0.006	0.05	3.76	0.452	Grp 4 Mean	30	284.72	291.78	47.6	1.588	0.030	13,560	
2,3,4,7,8-PeCDF	0.756	0.012	0.5	180	0.422	Grp 4 Mean	30	284.72	291.78	44.5	1.483	0.028	12,660	
1,2,3,4,7,8-HxCDF	0.518	0.019	0.1	17.5	0.307	Grp 4 Mean	30	284.72	291.78	32.3	1.079	0.020	9,210	
1,2,3,6,7,8-HxCDF	0.526	0.010	0.1	5.30	0.0892	Grp 4 Mean	30	284.72	291.78	9.40	0.313	0.006	2,676	
2,3,7,8-TCDF	0.275		0.1	12.2	0.673	14	30	282.83	294.77	71.4	2.380		20,190	10.59
1,2,3,7,8-PeCDF	0.219		0.05	4.34	0.452	14	30	282.83	294.77	47.9	1.598		13,560	10.59
2,3,4,7,8-PeCDF	0.757		0.5	197	0.422	14	30	282.83	294.77	44.8	1.492		12,660	10.59
1,2,3,4,7,8-HxCDF	0.526		0.1	19.5	0.307	14	30	282.83	294.77	32.6	1.085		9,210	10.59
1,2,3,6,7,8-HxCDF	0.532		0.1	5.84	0.0892	14	30	282.83	294.77	9.46	0.315		2,676	10.59
2,3,7,8-TCDF	0.284		0.1	11.6	0.673	23	30	291.87	296.23	69.2	2.306		20,190	10.19
1,2,3,7,8-PeCDF	0.216		0.05	4.00	0.452	23	30	291.87	296.23	46.5	1.549		13,560	10.19
2,3,4,7,8-PeCDF	0.741		0.5	181	0.422	23	30	291.87	296.23	43.4	1.446		12,660	10.19
1,2,3,4,7,8-HxCDF	0.506		0.1	17.7	0.307	23	30	291.87	296.23	31.6	1.052		9,210	10.19
1,2,3,6,7,8-HxCDF	0.522		0.1	5.36	0.0892	23	30	291.87	296.23	9.17	0.306		2,676	10.19
2,3,7,8-TCDF	0.276		0.1	9.91	0.673	39	30	288.72	298.82	69.9	2.331		20,190	9.93
1,2,3,7,8-PeCDF	0.220		0.05	3.51	0.452	39	30	288.72	298.82	47.0	1.566		13,560	9.93
2,3,4,7,8-PeCDF	0.804		0.5	176	0.422	39	30	288.72	298.82	43.8	1.462		12,660	9.93
1,2,3,4,7,8-HxCDF	0.559		0.1	17.4	0.307	39	30	288.72	298.82	31.9	1.063		9,210	9.93
1,2,3,6,7,8-HxCDF	0.560		0.1	5.13	0.0892	39	30	288.72	298.82	9.27	0.309		2,676	9.93
2,3,7,8-TCDF	0.266		0.1	8.47	0.673	43	30	281.14	277.93	71.8	2.394		20,190	8.54
1,2,3,7,8-PeCDF	0.206		0.05	3.04	0.452	43	30	281.14	277.93	48.2	1.608		13,560	8.54
2,3,4,7,8-PeCDF	0.771		0.5	160	0.422	43	30	281.14	277.93	45.0	1.501		12,660	8.54
1,2,3,4,7,8-HxCDF	0.542		0.1	15.8	0.307	43	30	281.14	277.93	32.8	1.092		9,210	8.54
1,2,3,6,7,8-HxCDF	0.538		0.1	4.70	0.0892	43	30	281.14	277.93	9.52	0.317		2,676	8.54
2,3,7,8-TCDF	0.266		0.1	9.51	0.673	53	30	279.06	291.15	72.4	2.412		20,190	12.23
1,2,3,7,8-PeCDF	0.212		0.05	3.66	0.452	53	30	279.06	291.15	48.6	1.620		13,560	12.23
2,3,4,7,8-PeCDF	0.755		0.5	182	0.422	53	30	279.06	291.15	45.4	1.512		12,660	12.23
1,2,3,4,7,8-HxCDF	0.500		0.1	17.1	0.307	53	30	279.06	291.15	33.0	1.100		9,210	12.23
1,2,3,6,7,8-HxCDF	0.514		0.1	5.29	0.0892	53	30	279.06	291.15	9.59	0.320		2,676	12.23

Table D-4. (cont.)

Analyte	Oil Reference 0.5X (Group 4)											
	Using Terminal BW			Fat Conc. (pg/g)	Fraction Retained in Liver FR <sub>liver</sub> (unitless)	FR <sub>liver</sub> S.D.	Fraction Retained in Fat FR <sub>fat</sub> (unitless)	FR <sub>fat</sub> S.D.	Fraction Retained Liver+Fat FR <sub>sum</sub> (unitless)	FR <sub>sum</sub> S.D.	WHO TEF (unitless)	Liver TEQ (pg/g)
	Liver Conc. (pg/g)	Fat Fraction (wa) (unitless)	Fat Weight (g)									
2,3,7,8-TCDF	206			118	0.109	0.016	0.130	0.014	0.239	0.030	0.1	
1,2,3,7,8-PeCDF	176			55.5	0.137	0.017	0.089	0.005	0.226	0.021	0.05	
2,3,4,7,8-PeCDF	830			53.4	0.681	0.077	0.091	0.005	0.772	0.080	0.5	
1,2,3,4,7,8-HxCDF	415			33.1	0.470	0.064	0.077	0.005	0.547	0.066	0.1	
1,2,3,6,7,8-HxCDF	125			9.17	0.494	0.067	0.073	0.003	0.568	0.067	0.1	
2,3,7,8-TCDF	196	0.0753	22.20	113	0.103		0.124		0.227		0.1	19.6
1,2,3,7,8-PeCDF	177	0.0753	22.20	54.5	0.138		0.089		0.227		0.05	8.85
2,3,4,7,8-PeCDF	744	0.0753	22.20	47.7	0.622		0.084		0.706		0.5	372
1,2,3,4,7,8-HxCDF	370	0.0753	22.20	29.1	0.425		0.070		0.496		0.1	37.0
1,2,3,6,7,8-HxCDF	115	0.0753	22.20	8.36 <i>J</i>	0.455		0.069		0.524		0.1	11.5
2,3,7,8-TCDF	208	0.0756	22.39	116	0.105		0.129		0.234		0.1	20.8
1,2,3,7,8-PeCDF	165	0.0756	22.39	51.4	0.124		0.085		0.209		0.05	8.25
2,3,4,7,8-PeCDF	838	0.0756	22.39	52.7	0.675		0.093		0.768		0.5	419
1,2,3,4,7,8-HxCDF	411	0.0756	22.39	31.7	0.455		0.077		0.532		0.1	41.1
1,2,3,6,7,8-HxCDF	123	0.0756	22.39	8.74 <i>J</i>	0.468		0.073		0.542		0.1	12.3
2,3,7,8-TCDF	182	0.0761	22.74	106	0.090		0.119		0.209		0.1	18.2
1,2,3,7,8-PeCDF	164	0.0761	22.74	53.0	0.120		0.089		0.209		0.05	8.2
2,3,4,7,8-PeCDF	807	0.0761	22.74	51.9	0.633		0.093		0.726		0.5	404
1,2,3,4,7,8-HxCDF	401	0.0761	22.74	33.6	0.432		0.083		0.515		0.1	40.1
1,2,3,6,7,8-HxCDF	113	0.0761	22.74	9.32 <i>J</i>	0.419		0.079		0.499		0.1	11.3
2,3,7,8-TCDF	227	0.0719	20.00	117	0.096		0.116		0.212		0.1	22.7
1,2,3,7,8-PeCDF	198	0.0719	20.00	58.8	0.125		0.087		0.211		0.05	9.90
2,3,4,7,8-PeCDF	941	0.0719	20.00	59.6	0.635		0.094		0.729		0.5	471
1,2,3,4,7,8-HxCDF	470	0.0719	20.00	37.9	0.436		0.082		0.518		0.1	47.0
1,2,3,6,7,8-HxCDF	144	0.0719	20.00	10.4 <i>J</i>	0.460		0.078		0.537		0.1	14.4
2,3,7,8-TCDF	219	0.0746	21.71	139	0.133		0.149		0.282		0.1	21.9
1,2,3,7,8-PeCDF	177	0.0746	21.71	59.9	0.160		0.096		0.256		0.05	8.85
2,3,4,7,8-PeCDF	819	0.0746	21.71	55.2	0.791		0.095		0.886		0.5	410
1,2,3,4,7,8-HxCDF	425	0.0746	21.71	33.3	0.564		0.079		0.643		0.1	42.5
1,2,3,6,7,8-HxCDF	130	0.0746	21.71	9.05 <i>J</i>	0.594		0.073		0.668		0.1	13.0

Table D-4. (cont.)

Analyte	Oil Reference 0.8X (Group 5)														
	Oil Reference 0.8X Mean Conc. (ng/mL)	Group 5 Rat IDs	Total Gavage Volume (mL)	Mean BW <sup>c</sup> (g)	Terminal BW (g)	Using Mean BW			Total Dose (pg)	Liver Weight (g)	Liver Conc. (pg/g)	Using Terminal BW			
						Total Dose[bw] <sup>b</sup> (pg/g)	Avg. Daily Dose[bw] <sup>a</sup> (pg/g)	Avg. Daily Dose[bw] <sup>a</sup> S.D.				Fat Weight Fraction (wa) (unitless)	Fat Weight (g)	Fat Conc. (pg/g)	
2,3,7,8-TCDF	1.023	Grp 5 Mean	30	267.09	272.60	115	3.831	0.078	30,690		357			154	
1,2,3,7,8-PeCDF	0.707	Grp 5 Mean	30	267.09	272.60	79.4	2.648	0.054	21,210		325			81.0	
2,3,4,7,8-PeCDF	0.642	Grp 5 Mean	30	267.09	272.60	72.1	2.404	0.049	19,260		1,614			80.1	
1,2,3,4,7,8-HxCDF	0.469	Grp 5 Mean	30	267.09	272.60	52.7	1.757	0.036	14,070		807			50.2	
1,2,3,6,7,8-HxCDF	0.136	Grp 5 Mean	30	267.09	272.60	15.3	0.509	0.010	4,080		247			13.8	
2,3,7,8-TCDF	1.023	15	30	264.87	276.11	116	3.862		30,690	10.19	327	0.0716	19.77	146	
1,2,3,7,8-PeCDF	0.707	15	30	264.87	276.11	80.1	2.669		21,210	10.19	295	0.0716	19.77	74.3	
2,3,4,7,8-PeCDF	0.642	15	30	264.87	276.11	72.7	2.424		19,260	10.19	1,450	0.0716	19.77	74.7	
1,2,3,4,7,8-HxCDF	0.469	15	30	264.87	276.11	53.1	1.771		14,070	10.19	734	0.0716	19.77	47.5	
1,2,3,6,7,8-HxCDF	0.136	15	30	264.87	276.11	15.4	0.513		4,080	10.19	228	0.0716	19.77	12.8 J	
2,3,7,8-TCDF	1.023	26	30	265.30	261.30	116	3.856		30,690	8.73	353	0.0686	17.94	143	
1,2,3,7,8-PeCDF	0.707	26	30	265.30	261.30	79.9	2.665		21,210	8.73	328	0.0686	17.94	81.2	
2,3,4,7,8-PeCDF	0.642	26	30	265.30	261.30	72.6	2.420		19,260	8.73	1,690	0.0686	17.94	85.5	
1,2,3,4,7,8-HxCDF	0.469	26	30	265.30	261.30	53.0	1.768		14,070	8.73	814	0.0686	17.94	53.6	
1,2,3,6,7,8-HxCDF	0.136	26	30	265.30	261.30	15.4	0.513		4,080	8.73	256	0.0686	17.94	13.4 J	
2,3,7,8-TCDF	1.023	27	30	261.50	262.98	117	3.912		30,690	8.63	372	0.069	18.14	154	
1,2,3,7,8-PeCDF	0.707	27	30	261.50	262.98	81.1	2.704		21,210	8.63	344	0.069	18.14	84.8	
2,3,4,7,8-PeCDF	0.642	27	30	261.50	262.98	73.7	2.455		19,260	8.63	1,750	0.069	18.14	84.5	
1,2,3,4,7,8-HxCDF	0.469	27	30	261.50	262.98	53.8	1.793		14,070	8.63	880	0.069	18.14	55.3	
1,2,3,6,7,8-HxCDF	0.136	27	30	261.50	262.98	15.6	0.520		4,080	8.63	268	0.069	18.14	17.2	
2,3,7,8-TCDF	1.023	29	30	276.09	280.73	111	3.705		30,690	9.13	377	0.0725	20.35	166	
1,2,3,7,8-PeCDF	0.707	29	30	276.09	280.73	76.8	2.561		21,210	9.13	355	0.0725	20.35	86.1	
2,3,4,7,8-PeCDF	0.642	29	30	276.09	280.73	69.8	2.325		19,260	9.13	1,630	0.0725	20.35	79.9	
1,2,3,4,7,8-HxCDF	0.469	29	30	276.09	280.73	51.0	1.699		14,070	9.13	834	0.0725	20.35	48.9	
1,2,3,6,7,8-HxCDF	0.136	29	30	276.09	280.73	14.8	0.493		4,080	9.13	247	0.0725	20.35	13.3 J	
2,3,7,8-TCDF	1.023	36	30	267.72	281.86	115	3.821		30,690	10.03	355	0.0727	20.50	162	
1,2,3,7,8-PeCDF	0.707	36	30	267.72	281.86	79.2	2.641		21,210	10.03	305	0.0727	20.50	78.5	
2,3,4,7,8-PeCDF	0.642	36	30	267.72	281.86	71.9	2.398		19,260	10.03	1,550	0.0727	20.50	76.1	
1,2,3,4,7,8-HxCDF	0.469	36	30	267.72	281.86	52.6	1.752		14,070	10.03	773	0.0727	20.50	45.8	
1,2,3,6,7,8-HxCDF	0.136	36	30	267.72	281.86	15.2	0.508		4,080	10.03	235	0.0727	20.50	12.2 J	

**Table D-4. (cont.)**

Oil Reference 0.8X (Group 5)								
Analyte	Fraction Retained in Liver FR <sub>liver</sub> (unitless)	FR <sub>liver</sub> S.D.	Fraction Retained in Fat FR <sub>fat</sub> (unitless)	FR <sub>fat</sub> S.D.	Fraction Retained Liver+Fat FR <sub>sum</sub> (unitless)	FR <sub>sum</sub> S.D.	WHO TEF (unitless)	Liver TEQ (pg/g)
2,3,7,8-TCDF	0.109	0.007	0.099	0.013	0.208	0.019	0.1	
1,2,3,7,8-PeCDF	0.143	0.007	0.074	0.007	0.218	0.014	0.05	
2,3,4,7,8-PeCDF	0.778	0.020	0.080	0.003	0.859	0.021	0.5	
1,2,3,4,7,8-HxCDF	0.532	0.020	0.068	0.002	0.600	0.020	0.1	
1,2,3,6,7,8-HxCDF	0.562	0.014	0.062	0.003	0.624	0.014	0.1	
2,3,7,8-TCDF	0.109		0.094		0.203		0.1	32.7
1,2,3,7,8-PeCDF	0.142		0.069		0.211		0.05	14.75
2,3,4,7,8-PeCDF	0.767		0.077		0.844		0.5	725
1,2,3,4,7,8-HxCDF	0.532		0.067		0.598		0.1	73.4
1,2,3,6,7,8-HxCDF	0.569		0.062		0.631		0.1	22.8
2,3,7,8-TCDF	0.100		0.084		0.184		0.1	35.3
1,2,3,7,8-PeCDF	0.135		0.069		0.204		0.05	16.4
2,3,4,7,8-PeCDF	0.766		0.080		0.846		0.5	845
1,2,3,4,7,8-HxCDF	0.505		0.068		0.573		0.1	81.4
1,2,3,6,7,8-HxCDF	0.548		0.059		0.607		0.1	25.6
2,3,7,8-TCDF	0.105		0.091		0.196		0.1	37.2
1,2,3,7,8-PeCDF	0.140		0.073		0.212		0.05	17.2
2,3,4,7,8-PeCDF	0.784		0.080		0.864		0.5	875
1,2,3,4,7,8-HxCDF	0.540		0.071		0.611		0.1	88
1,2,3,6,7,8-HxCDF	0.567		0.076		0.643		0.1	26.8
2,3,7,8-TCDF	0.112		0.110		0.222		0.1	37.7
1,2,3,7,8-PeCDF	0.153		0.083		0.235		0.05	17.75
2,3,4,7,8-PeCDF	0.773		0.084		0.857		0.5	815
1,2,3,4,7,8-HxCDF	0.541		0.071		0.612		0.1	83.4
1,2,3,6,7,8-HxCDF	0.553		0.066		0.619		0.1	24.7
2,3,7,8-TCDF	0.116		0.108		0.224		0.1	35.5
1,2,3,7,8-PeCDF	0.144		0.076		0.220		0.05	15.25
2,3,4,7,8-PeCDF	0.807		0.081		0.888		0.5	775
1,2,3,4,7,8-HxCDF	0.551		0.067		0.618		0.1	77.3
1,2,3,6,7,8-HxCDF	0.578		0.061		0.639		0.1	23.5

**Note:** J – The amount detected is below the Lower Calibration Limit of the instrument.

<sup>a</sup> Mean of body weights from study days 1, 7, 15, 22, and 30

**Table D-5. Tissue concentrations in control group composite samples**

Analyte	Group 1 Composite Feed Control		Group 2 Composite Oil Control	
	Liver (pg/g)	Fat (pg/g)	Liver (pg/g)	Fat (pg/g)
2,3,7,8-TCDD	0.172 <i>U</i> <sup>a</sup>	0.298 <i>J</i>	0.193 <i>U</i> <sup>a</sup>	0.283 <i>U</i> <sup>a</sup>
1,2,3,7,8-PeCDD	0.768 <i>J</i>	0.642 <i>J</i>	0.824 <i>J</i>	0.518 <i>J</i>
1,2,3,4,7,8-HxCDD	0.358 <i>J</i>	0.232 <i>U</i>	0.396 <i>J</i>	0.200 <i>U</i>
1,2,3,6,7,8-HxCDD	1.04 <i>J</i>	0.365 <i>J</i>	1.31 <i>J</i>	0.326 <i>J</i>
1,2,3,7,8,9-HxCDD	0.554 <i>J</i>	0.208 <i>J</i>	0.606 <i>J</i>	0.206 <i>J</i>
1,2,3,4,6,7,8-HpCDD	5.24	0.832 <i>J</i>	6.54	0.836 <i>J</i>
OCDD	17.6 <i>B</i>	2.56 <i>J,B</i>	23.6 <i>B</i>	2.45 <i>J,B</i>
2,3,7,8-TCDF	0.724 <i>J</i>	0.539 <i>J</i>	0.728 <i>J</i>	0.472 <i>J</i>
1,2,3,7,8-PeCDF	0.127 <i>U</i> <sup>a</sup>	0.234 <i>U</i>	0.166 <i>U</i> <sup>a</sup>	0.260 <i>U</i>
2,3,4,7,8-PeCDF	1.13 <i>J</i>	0.235 <i>U</i>	1.41 <i>J</i>	0.274 <i>U</i>
1,2,3,4,7,8-HxCDF	0.375 <i>J</i>	0.0810 <i>U</i>	0.534 <i>J</i>	0.0665 <i>U</i>
1,2,3,6,7,8-HxCDF	0.374 <i>J</i>	0.0778 <i>U</i>	0.374 <i>J</i>	0.0613 <i>U</i>
2,3,4,6,7,8-HxCDF	0.277 <i>J</i>	0.0868 <i>U</i>	0.282 <i>J</i>	0.0726 <i>U</i>
1,2,3,7,8,9-HxCDF	0.0838 <i>U</i>	0.132 <i>U</i>	0.0693 <i>U</i>	0.105 <i>U</i>
1,2,3,4,6,7,8-HpCDF	1.85 <i>J</i>	0.278 <i>U</i>	2.83 <i>J</i>	0.242 <i>J</i>
1,2,3,4,7,8,9-HpCDF	0.194 <i>J</i>	0.349 <i>U</i>	0.270 <i>J</i>	0.241 <i>U</i>
OCDF	1.34 <i>J</i>	0.483 <i>U</i>	2.42 <i>J</i>	0.463 <i>U</i>
<b>TEQ<sup>b</sup></b>	<b>1.96</b>	<b>1.26</b>	<b>2.26</b>	<b>1.12</b>

**Note:** *B* – This compound was also detected in the method blank.

*J* – The amount detected is below the Lower Calibration Limit of the instrument.

*U* – Not detected; value represents the sample-specific detection limit, unless noted otherwise.

<sup>a</sup> Nondetect reported to the EMPC (Estimated Maximum Possible Concentration).

<sup>b</sup> Toxicity equivalence concentration (TEQ) calculated using the World Health Organization (WHO) toxicity equivalence factors.

**Table D-6. Rat liver microsomal EROD activities**

Entrix Sample ID	Exponent Group	Exponent Rat ID	Statistics			Group Statistics	
			Mean	SD	CV (%)	Mean	SD
S17	Gp-1	17	31.5	0.43	1.37	33.2	6.13
S18	Gp-1	18	25.4	0.82	3.21		
S20	Gp-1	20	32.3	0.38	1.18		
S44	Gp-1	44	34.1	0.40	1.17		
S46	Gp-1	46	42.4	2.29	5.40		
S32	Gp-2	32	33.5	1.04	3.10	40.6	7.15
S35	Gp-2	35	33.4	0.88	2.64		
S38	Gp-2	38	44.2	0.45	1.01		
S40	Gp-2	40	49.9	0.85	1.69		
S54	Gp-2	54	42.2	0.88	2.10		
S22	Gp-3	22	42.3	1.25	2.95	53.6	8.07
S24	Gp-3	24	49.3	1.38	2.79		
S37	Gp-3	37	54.3	0.52	0.95		
S47	Gp-3	47	61.2	1.99	3.25		
S50	Gp-3	50	61.2	0.99	1.62		
S14	Gp-4	14	73.3	2.52	3.43	80.8	17.9
S23	Gp-4	23	83.6	4.53	5.42		
S39	Gp-4	39	109.9	8.26	7.52		
S43	Gp-4	43	74.7	2.03	2.71		
S53	Gp-4	53	62.6	1.91	3.05		
S15	Gp-5	15	115.1	4.84	4.21	106.4	16.6
S26	Gp-5	26	119.8	4.04	3.37		
S27	Gp-5	27	116.8	6.76	5.79		
S29	Gp-5	29	100.1	3.54	3.53		
S36	Gp-5	36	80.0	3.55	4.43		
S30	Gp-6	30	82.0	1.89	2.31	110.1	24.1
S41	Gp-6	41	118.2	4.67	3.95		
S42	Gp-6	42	142.9	8.34	5.84		
S48	Gp-6	48	116.3	0.73	0.63		
S51	Gp-6	51	91.1	1.00	1.09		

**Note:** SD – standard deviation  
CV – coefficient of variability

**Table D-7. Rat liver microsomal MROD activities**

Entrix Sample ID	Exponent Group	Exponent Rat ID	Statistics			Group Statistics	
			Mean	SD	CV (%)	Mean	SD
S17	Gp-1	17	26.9	2.26	8.18	25.7	2.2
S18	Gp-1	18	27.7	1.03	3.76		
S20	Gp-1	20	26.6	1.08	3.87		
S44	Gp-1	44	22.0	1.05	4.89		
S46	Gp-1	46	25.3	1.05	2.89		
S32	Gp-2	32	29.3	1.18	4.09	26.9	1.8
S35	Gp-2	35	26.5	2.16	8.14		
S38	Gp-2	38	24.4	0.30	1.09		
S40	Gp-2	40	26.9	1.63	6.22		
S54	Gp-2	54	27.5	1.43	5.12		
S22	Gp-3	22	36.3	1.56	4.56	33.3	3.6
S24	Gp-3	24	36.1	2.27	6.42		
S37	Gp-3	37	34.8	2.47	7.26		
S47	Gp-3	47	31.5	1.87	5.94		
S50	Gp-3	50	28.0	0.97	3.50		
S14	Gp-4	14	31.3	1.30	4.29	34.9	10.0
S23	Gp-4	23	51.2	0.30	0.38		
S39	Gp-4	39	36.5	0.58	1.56		
S43	Gp-4	43	24.8	1.36	5.52		
S53	Gp-4	53	30.9	1.76	5.65		
S15	Gp-5	15	52.0	2.78	5.24	41.9	7.4
S26	Gp-5	26	36.6	2.40	6.58		
S27	Gp-5	27	39.3	1.32	3.54		
S29	Gp-5	29	47.0	3.59	7.68		
S36	Gp-5	36	34.5	2.62	7.61		
S30	Gp-6	30	33.6	2.67	7.97	34.5	5.5
S41	Gp-6	41	39.0	1.02	2.50		
S42	Gp-6	42	30.1	1.43	4.70		
S48	Gp-6	48	41.2	1.93	4.68		
S51	Gp-6	51	28.7	1.37	4.86		

**Note:** SD – standard deviation  
CV – coefficient of variability



**Table D-8. Summary of relative bioavailability estimates for the follow-up rat study**

Analyte	Fraction of Administered Dose Retained									RBA Estimates					
	Liver			Adipose			Liver + Adipose			Liver		Adipose		Liver + Adipose	
	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	C.V.	Mean	C.V.	Mean	C.V.
<b>Tittabawassee River Floodplain Soil (Group 6)</b>															
2,3,7,8-TCDF	0.065	0.006	10%	0.065	0.007	11%	0.130	0.012	9%						
1,2,3,7,8-PeCDF	0.079	0.008	11%	0.044	0.003	8%	0.123	0.011	9%						
2,3,4,7,8-PeCDF	0.432	0.035	8%	0.049	0.004	7%	0.481	0.037	8%						
1,2,3,4,7,8-HxCDF	0.295	0.022	7%	0.042	0.005	12%	0.337	0.026	8%						
1,2,3,6,7,8-HxCDF	0.331	0.030	9%	0.048	0.007	15%	0.379	0.035	9%						
<b>Oil Reference 0.2X (Group 3)</b>															
2,3,7,8-TCDF	0.118	0.012	11%	0.155	0.006	4%	0.273	0.009	3%	<b>Soil vs. Oil Reference 0.2X</b>					
1,2,3,7,8-PeCDF	0.123	0.009	7%	0.091	0.004	4%	0.213	0.006	3%	55%	14%	42%	11%	48%	10%
2,3,4,7,8-PeCDF	0.656	0.010	2%	0.100	0.011	11%	0.756	0.012	2%	65%	13%	49%	9%	58%	9%
1,2,3,4,7,8-HxCDF	0.435	0.016	4%	0.083	0.010	12%	0.518	0.019	4%	66%	8%	49%	13%	64%	8%
1,2,3,6,7,8-HxCDF	0.446	0.009	2%	0.081	0.006	8%	0.526	0.010	2%	68%	8%	51%	17%	65%	9%
										74%	9%	60%	17%	72%	9%
<b>Oil Reference 0.5X (Group 4)</b>															
2,3,7,8-TCDF	0.109	0.016	15%	0.130	0.014	11%	0.239	0.030	13%	<b>Soil vs. Oil Reference 0.5X</b>					
1,2,3,7,8-PeCDF	0.137	0.017	12%	0.089	0.005	5%	0.226	0.021	10%	59%	18%	50%	15%	54%	16%
2,3,4,7,8-PeCDF	0.681	0.077	11%	0.091	0.005	6%	0.772	0.080	10%	58%	16%	49%	9%	55%	13%
1,2,3,4,7,8-HxCDF	0.470	0.064	14%	0.077	0.005	7%	0.547	0.066	12%	63%	14%	54%	9%	62%	13%
1,2,3,6,7,8-HxCDF	0.494	0.067	14%	0.073	0.003	5%	0.568	0.067	12%	63%	16%	54%	14%	62%	14%
										67%	16%	65%	16%	67%	15%
<b>Oil Reference 0.8X (Group 5)</b>															
2,3,7,8-TCDF	0.109	0.007	6%	0.099	0.013	13%	0.208	0.019	9%	<b>Soil vs. Oil Reference 0.8X</b>					
1,2,3,7,8-PeCDF	0.143	0.007	5%	0.074	0.007	9%	0.218	0.014	6%	59%	11%	66%	17%	62%	13%
2,3,4,7,8-PeCDF	0.778	0.020	3%	0.080	0.003	4%	0.859	0.021	2%	55%	12%	59%	12%	57%	11%
1,2,3,4,7,8-HxCDF	0.532	0.020	4%	0.068	0.002	3%	0.600	0.020	3%	55%	8%	61%	8%	56%	8%
1,2,3,6,7,8-HxCDF	0.562	0.014	2%	0.062	0.003	5%	0.624	0.014	2%	55%	8%	62%	12%	56%	8%
										59%	9%	77%	16%	61%	10%

**Notes:** RBA – relative bioavailability, calculated as: Fraction of administered dose retained<sub>test material</sub> / Fraction of administered dose retained<sub>reference material</sub>

S.D. – standard deviation

C.V. – coefficient of variability

For fraction of administered dose retained: C.V. = Standard Deviation / Mean

For RBA estimates: C.V. =  $(CV_{\text{soil}}^2 + CV_{\text{reference}}^2)^{0.5}$